Investigating the environmental partitioning of microplastics in two contrasting marine ecosystems.

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

Plastic is a pervasive pollutant of marine ecosystems globally, found throughout the water column, in sediments, and biota. Small plastic particles, or microplastics, are numerous and readily ingested by marine organisms. However, these plastic particles are distributed unevenly throughout marine environments and the physical properties of the particle can influence how they are transported, and ultimately where they are found. In this thesis I review the current literature to explain how plastic particles behave in the marine environment according to their physical attributes, and how this might influence the number and types of plastic to which organisms are exposed. I then explore two cases of plastic partitioning across compartments of an Arctic fjord (Kongsfjorden, Svalbard), and in rocky shore habitats of Devon and Cornwall, UK via an extensive field sampling campaign.

Using a boat based sampling programme, seawater microplastic contamination for two different water bodies, local Arctic and Atlantic, within an Arctic fjord was assessed via sampling at two different depths of the water column. Salinity-temperature-depth (CTD) profiles were acquired, and microplastic particles collected from sea surface and 160 m depth at three different locations in Kongsfjorden, Svalbard, using surface plankton net trawls and niskin bottles. The isolated microplastic particles were counted and analysed by FTIR spectroscopy. The parameters defining Atlantic water were not detected, however the mean microplastic concentration in deep waters (2.9 (± 1.7) x 10^4 particles m^{-3}) was significantly greater than surface waters (112 ± 53 particles m^{-3}). The most common polymers identified were polyester (18%), ethylene-propylene copolymer (11.8 %), and polyacrylic acid and polyethylene (10 % each). Particles at the surface were significantly larger than particles at 160 m, fragments were on average 5430 µm larger, and fibres 850 µm longer. Significantly greater proportions of white fragments and blue fibres were found at the surface compared to 160 m, and black and blue fragments at 160 m compared to surface water.

The environmental partitioning of macro-, meso- and microplastics across surface sediment, seawater, and mussels *Mytilus edulis* were then analysed from

9 intertidal locations in the South West of England. Micro- and mesoplastic-like particles were found in 88.5% of the 269 mussels sampled, ranging from 1.43 to 7.64 items per mussel. Of these plastic particles, 70.9% were identified as semi-synthetic (mainly modified-cellulose). Mussel microplastic abundance, but not polymer type, was correlated with that of their surrounding sediment, but not with sea-surface microplastic concentration or mussel size. Significant differences were present in the relative abundance of polymers and particle sizes between seawater, sediment, and mussels, with mussels containing a greater abundance of cellulose fibre but less polyvinyl polymer. The particle characteristics of mussel microplastic contamination are not directly proportional to that of the microplastics in their surrounding environment.

The data from these two contrasting ecosystems both add to the growing evidence that microplastics are not just a sea surface problem and partition across marine ecosystems with particle characteristics such as polymer type (density), shape, and size all likely playing a role. Although uptake of particles by organisms may be subject to processes of selection, ultimately, the distribution of plastic particles governs the particles to which organisms are exposed and might ingest. Therefore, understanding particle characteristics and dynamics will play a role in determining the biological consequences of microplastic pollution in marine biota.

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Definitions/abbreviations

PE: Polyethylene

PP: Polypropylene

PS: Polystyrene

PET: Polyethylene terephthalate

PVC: Polyvinyl chloride

HDPE: High-density polyethylene

LDPE: Low-density polyethylene

LW: Local water

AW: Atlantic water

IW: Intermediate water

ArW: Arctic water

WCW: Winter cooled water

TAW: Transformed Atlantic water

FTIR: Fourier-transform infrared

GPGP: Great Pacific garbage patch

KOH: Potassium hydroxide

HNO_3: Nitric acid

$ZnCl_2$: Zinc chloride

SMI: Sediment-Microplastic Isolation (unit)

Chapter 1

Literature review - Factors influencing the distribution of microplastics in the marine environment and implications for uptake in organisms.

The issue of plastic pollution in the marine environment and single-use plastic waste has now become something of a cultural phenomenon. With far-reaching public and social media attention, the extent of plastic pollution research has never been greater. Plastic constitutes a variety of synthetic organic polymers of petrochemical origin. Due to their low cost of manufacture, durability, and versatility, in the short period of time since the first manufactured plastics they have grown to encompass nearly all aspects of modern life from packaging of food and goods, to creation of consumer products. The production of plastic has increased rapidly in the 21st century, with an estimated 8300 million metric tonnes of plastic created to date, and a compound annual growth rate of 8.4 % between 1950 and 2015 (Geyer, Jambeck and Law, 2017). Global plastic production was almost 350 million tonnes in the year 2017 (PlasticsEurope, 2018). The combination of manufacturing of throw-away, "single-use" plastics, and poor waste management of plastic items has resulted in an estimated 4.8 to 12.7 million metric tonnes of plastic items entering the marine environment each year (Jambeck *et al.*, 2015). This originates from coastal populations and waste management sites, accidental losses at sea such as shipping containers and lost fishing equipment, or "urban waste spill-over" transported from urban environments by rivers (Jambeck *et al.*, 2015; Unger and Harrison, 2016; Lebreton *et al.*, 2017).

To understand the risks that microplastics pose requires an understanding of how marine organisms encounter particles in their immediate habitats, yet our knowledge of how microplastics behave in complex ecosystems remains limited. A variety of biotic and abiotic processes such as prevailing winds and currents, tides, weather patterns, location, local sources, interactions with marine life, and the characteristics of the plastic particles are all likely to play a role. Here I will begin by reviewing what is known about how these complex factors interact with marine ecosystems to determine partitioning and hence availability to organisms.

As the ecological and physiological factors that may determine the chance of uptake by organisms are also complex, I will then go on to review our current knowledge on the factors influencing the likelihood of uptake by marine organisms.

1.1. Plastic as a marine pollutant

Microplastics are most frequently defined as particles of plastic polymer < 5 mm in size, although as the field of microplastic research has developed there have been calls for a standardized and more rigorous set of definitions for plastic pollution (Frias and Nash, 2019; Hartmann *et al.*, 2019). Hartmann *et al.* (2019) suggest 1 to < 1000 µm as the size criterion for microplastic. Plastic may break down into nano-scale fragments which have been observed in the North Atlantic subtropical gyre (Halle *et al.*, 2017). In terms of their origin, plastic particles can be categorised into either primary or secondary microplastic.

Primary microplastic particles are directly manufactured before unintentionally reaching the marine environment. Examples include pre-production plastic pellets (nurdles or “mermaids tears”), synthetic fibres from textiles, tyres, road markings, marine coatings, abrasive microplastic beads in cosmetics products, and city dust (Friot and Boucher, 2017). Sources of primary microplastics include direct losses at sea, waste water and road run-off into rivers, and atmospheric deposition (Auta, Emenike and Fauziah, 2017; Friot and Boucher, 2017; Windsor *et al.*, 2019). City dust can be transported by wind or water and is a known source of microplastics in the marine environment (Dris *et al.*, 2016). Rainfall and high levels of precipitation can cause increased urban run-off of plastic debris and fragmented synthetic polymers such as rubber from vehicle tyres and thermoplastic road markings. Recent research suggests a strong local influence of primary microplastics on the coastline which correlates to distance from river outputs, however the exact total input of primary microplastic into the marine environment is difficult to evaluate. However, it is estimated that between 0.8 - 2.5 million tons of primary microplastics are released into the oceans per year, with total global losses to the environment estimated at 1.8 - 5.0 million tons per year (Friot and Boucher, 2017).

Secondary microplastic is generated by the degradation of larger plastic items already in the marine environment. The conditions of beach environments are particularly conducive of the degradation of plastic debris, where a combination of physical, chemical, and biological processes contribute to the weakening of bonds in plastic polymers and their gradual fragmentation into smaller pieces (Corcoran, Biesinger and Grifi, 2009). The major process of degradation is photo-oxidative (the action of solar UV radiation); other processes include biological breakdown by organisms, thermo-oxidative degradation (slow oxidative breakdown at moderate temperatures), and physical stresses such as tidal and wave action at the coast (Andrady, 2011; Hodgson, Bréchon and Thompson, 2018). Examples of biodegradation include the feeding activity of amphipods *Orchestia gammarellus* which can result in the fragmentation of polyethylene plastic carrier bags, and the grazing activity of sea urchins *Paracentrotus lividus* which produce microplastic fragments from macroplastic (Hodgson, Bréchon and Thompson, 2018; Porter, Smith and Lewis, 2019). When considering values from Jambeck *et al.* (2015) and Friot and Boucher (2017) who estimate "secondary" plastic releases of 4.8 - 12.7 million tonnes per year, and 3.2 million tonnes per year, respectively, then secondary microplastic might contribute between ~ 60 - 80 % of released microplastic by mass.

The term "microplastic" is used to describe a variety of particulate pollution and a complex mixture of synthetic polymers that might be better described as "anthropogenic micro-litter". An example is the inclusion of modified cellulose fibres in microplastic studies, a commonly observed particle often comprising a large proportion of observed particles. These are considered semi-synthetic due to the chemical processing of the fibres and addition of artificial dyes during the production of artificial textile products such as rayon, however individual fibres are challenging to differentiate from unadulterated cellulose fibres from plants such as cotton, sisal, and jute (Remy *et al.*, 2015; H. Li *et al.*, 2018; Cai *et al.*, 2019). Marine microplastic predominantly comprises pellets, fragments, and fibres but also contains a variety of other shape classes that may be somewhat subjective such as flakes, filaments, sponges, foams, ropes, rubber, line, sheet, films, and microbeads (Ivar Do Sul and Costa, 2014; Frias *et al.*, 2018; Frias and Nash, 2019).

The physical properties of marine plastic particles are varied in chemical composition and constituent polymers, shape and structure, solid state and solubility, colour, and chemical additives (Hartmann *et al.*, 2019). The relative difference in size between the smallest and largest microplastic is great, from 1 µm up to 5000 µm, or up to 1000 µm as recommended by Hartmann *et al.* (2019). There is even a broad range of physical characteristics within the plastic resins included in discrete polymer categories (e.g. “polyethylene”) in terms of density, crystallinity, and tensile strength, and the blends of polymers plastic manufacturers incorporate into products (Andrady, 2017). Plastic can include functional additives such as curing and foaming agents, and biocides. Common functional additives include phthalates which are plasticizers for softening plastic, brominated flame retardants to reduce flammability, nonylphenol as an antioxidant and sometimes plasticizer, and Bisphenol A (BPA) which is a monomer in polycarbonate and epoxy resins. The contribution of these chemical compounds can be significant, phthalates can make up 10 - 60 % of polyvinyl chloride (PVC) by weight, and plastic can also include colourants, fillers (e.g. clay, mica, talc), and reinforcements (e.g. glass fibres, carbon fibres) (Hansen *et al.*, 2013; Hermabessiere *et al.*, 2017).

This diverse nature of the particles that comprise microplastic pollution makes assessing their impact on organisms challenging. The issue is knowing the types and number of particles organisms are actually exposed to. Common practice is to refer to sea surface concentration measurements, and values of plastic production assuming they enter the marine environment and are biologically accessible in similar proportions. The most common non-fibre plastics produced are polyethylene (36 %) polypropylene (21 %) and polyvinyl chloride (12 %) followed by polyethylene terephthalate, polyurethane, and polystyrene (< 10 % each) (Geyer, Jambeck and Law, 2017). PE and PP are the most commonly observed polymers in some cases such as the surface of the open sea and beached macro-scale debris on the coast (Corcoran, Biesinger and Grifi, 2009; Morét-ferguson *et al.*, 2010; Pedrotti *et al.*, 2016). However, this is not a consistent pattern across all size scales and locations. Once released into the marine environment numerous biotic and abiotic factors influence the fates of

microplastic particles resulting in a complex distribution across marine compartments over time, these factors I will discuss below.

1.2. Distribution of plastic particles in the marine environment

Microplastic particles are found in almost every aquatic region from the Arctic to the tropics, and in almost all zones and habitats from the sea surface and pelagic to the benthos and the deep sea (e.g. Cozar *et al.*, 2014; Hazimah, Nor and Obbard, 2014; Woodall *et al.*, 2014; Peeken *et al.*, 2018). The total global mass of floating microplastic in the world's oceans is estimated at 15 - 51 trillion particles, weighing a total of between 9.3×10^3 and 2.4×10^5 metric tonnes (Van Sebille *et al.*, 2015). Although Everaert *et al* (2018) estimated a higher estimated global mass of 4.9×10^5 tonnes of microplastic, based on historical annual plastic production and projected growth, export to sediments, and mass loss to solar radiation and oxygenation. By the year 2100, the total mass of floating microplastics is predicted to increase to between 2.5×10^7 and 1.3×10^8 tonnes (best and worst case scenario, respectively) (Everaert *et al.*, 2018). The estimated accumulated mass of sea surface microplastic particles (as of 2014) is thought to account for a very small proportion (~ 1 %) of the global plastic released into the marine environment each year (as of 2010) (Van Sebille *et al.*, 2015). Floating plastic debris can travel long distances eventually beaching on the shore and littering our coastlines or sinking to be incorporated into seafloor sediment (Lebreton and Borrero, 2013; Nelms *et al.*, 2017; Porter *et al.*, 2018). We are increasingly understanding the factors influencing movement of microplastic particles and how this influences their distribution throughout the marine environment.

1.2.1. Inputs

A major pathway of plastic debris to the marine environment is rivers, which release an estimated 1.15 - 2.41 million tonnes of plastic into the oceans each year (Lebreton *et al.,* 2017). River beds suffer from extensive microplastic contamination, particularly in urban areas which may contain as many as 517,000

particles per m^2 (River Tame, UK), but are likely only a temporary sink for microplastics before they move into the oceans (Hurley, Woodward and Rothwell, 2018). In Asia, notably China and India, which represents 86 % of predicted global river plastic mass input, the peak input of plastic is correlated with the Summer monsoons, and in Europe river plastic inputs peak between November and May (Lebreton *et al.*, 2017). Export from river beds is influenced by water flow and flooding events, an investigation of ten rivers in North-West England revealed major changes to the structure of the microplastic contamination of fluvial sediment after flooding with the typical change being a decrease in concentration, from a mean of 6,350 to 2,812 particles kg^{-1} (Hurley, Woodward and Rothwell, 2018). Along the Chennai coastline, Veerasingam *et al.* (2016) reported a three-fold increase in microplastic abundance after the 2015 South India flood event; particles collected pre-flood exhibited high levels of degradation through loss of material, colour loss, biofouling, surface erosion and cracking, whereas microplastics collected post-flood were mostly virgin particles suggesting import of newer microplastic.

Yu *et al.* (2018) used computer modelling and field observations to show that the presence of large rivers was indicative of the abundance of microplastic particles in coastal sediment on the SE coast of the US. This relationship is also true for the Hong Kong area where two independent studies observed the highest concentrations of microplastic in close proximity to the mouth of the Pearl River (Fok and Cheung, 2015; Lo *et al.*, 2018). They also found a weak positive correlation ($R^2 = 0.43$) between microplastic abundance and the extent of nearby urbanization, a similar finding to Pedrotti *et al* (2016) who found plastic concentrations were significantly correlated with closeness to coastal human population.

An additional clue to the riverine origin of some coastal microplastics is the large number of fibres found in coastal sediments and their similarity to particles found in domestic greywater, these are typically cellulose or synthetic polymers such as polyester and polyethylene terephthalate (PET) (Yu *et al.*, 2016, 2018; Lo *et al.*, 2018; Raju *et al.*, 2018). These fibrous particles are commonly associated with the textiles industry and the result of washing clothes, a single garment can release > 1900 particles in a single wash (Browne *et al.*, 2011). Despite relatively

effective water cleaning processes which can in some instances remove up to 99.9% of all microplastics, a small proportion is released into rivers (Carr, Liu and Tesoro, 2016). In the river Irwell (Manchester, UK) plastic microbeads in river sediment, likely from cosmetic products, increased in association with areas influenced by release of waste water effluent by sewage treatment plants and combined sewer overflows (Murphy *et al.*, 2016; Kalčíková *et al.*, 2017; Hurley, Woodward and Rothwell, 2018). Reported particle concentrations for waste water treatment plant effluents are varied, from one particle per 1.14 thousand litres of final effluent, to one particle per litre (Raju *et al.*, 2018). Unfortunately, even the plastic particles removed by wastewater treatment sedimentation processes may one day return to oceans. The sewage sludge or biosolids containing microplastics are often recycled by application to soils as fertiliser or disposed of in landfill; the retention of “heavier-than-water” microplastic in soils has been estimated at approximately 16 - 38 %, the remainder will diffuse out of the soil and may ultimately return to river systems and then the marine ecosystem albeit in a less direct route (Clarke and Smith, 2011; Nizzetto *et al.*, 2016; Mintenig *et al.*, 2017).

1.2.2. Horizontal/surface movement

Plastic particles are carried on oceanic currents resulting in accumulation or retention by circulating oceanic gyres; arguably the most studied areas in terms of plastic research these regions offer some of the longest timescales for observations in plastic trends in the open sea (Cozar *et al.*, 2014; Cózar *et al.*, 2017). Numerous reports show that sea surface plastic is unevenly distributed, often occurring in distinct regions of accumulation (e.g. Eriksen *et al.*, 2013; Cozar *et al.*, 2014; Lebreton *et al.*, 2018). A comprehensive review of floating plastic debris by Van Sebille *et al.* (2015) compiled a standardized global dataset including data from 11,854 surface trawls carried out between 1971 and 2013, and used three different ocean circulation models to spatially interpolate these observations (Van Sebille *et al.*, 2015). In the open ocean, the study identified subtropical gyres of the North Atlantic and North Pacific as regions with the highest concentration of microplastics, containing 10^8 particles km^{-2}. Subtropical gyres, such as the “Great Pacific Garbage Patch” receive particular media

attention, however this study excluded the Arctic, a region from which concentrations are reported to be as high as 3.2 x 10^5 items km^{-2} in the Arctic sector of the Greenland and Barents seas (Cózar *et al.*, 2017).

Open ocean regions beyond accumulation zones typically contain far fewer microplastic particles, sometimes down to 0 detectable particles per km^2, however the methods of detection of are limited by the use of relatively large gauge net sizes and the number of small microplastic particles in the marine environment is likely to be underestimated. Plastic sampling net sizes typically range from 0.15 to 3.0 mm, with over 90 % of data collected by manta or neuston nets of 0.333 or 0.335 mm (Van Sebille *et al.,* 2015). While some field studies utilise finer mesh sizes in most studies a large proportion of microplastic particles in water are left uncaptured. In samples of Arctic sea ice 67 % of particles were within the smallest observable size category at 11 µm, and the majority of particles were well below the mesh size of a standard neuston sampling net (Cózar *et al.*, 2017). In some cases whole water sampling may be the more appropriate method, shown to collect over three orders of magnitude more microplastic per volume of water as well as a greater size range and greater proportion of non-fibrous plastic particles than a 335 µm neuston net, however this comes at the cost of sampling a much smaller volume of water (Barrows *et al.*, 2017).

The main driving force of plastic accumulation at sea surface is wind stress which results in the major horizontal oceanic circulation patterns (Maximenko *et al.*, 2009). Prevailing winds in the North Pacific result in two anticyclonic circulation patterns, a sub-polar and a sub-tropical gyre. The Eastern garbage patch between Hawaii and the US West coast, which is more commonly known as the Great Pacific Garbage Patch (GPGP), is the location of an atmospheric high pressure zone where positive wind stress curl lead to a “dead-zone” for currents (Howell *et al.*, 2012). Observations from an 11-year data set confirm spatial patterns of plastic in this region to have a strong dependence on upper ocean circulating currents, where accumulation is associated with subtropical convergence in surface currents generated by wind-driven Ekman transport and geostrophic circulation (Maximenko *et al.*, 2009; Law *et al.*, 2014). Previously, median concentrations of 3.3 x 10^4 pieces km^{-2} within the accumulation zone, and

0 pieces km^{-2} outside have been reported (Law *et al.*, 2014). More recently, surface manta trawls and aerial photography show that by abundance microplastics and mesoplastics are the most numerous size class with mean concentrations of 678,000 and 22,000 pieces km^{-2} (respectively) measured inside the GPGP which decrease by orders of magnitude with increasing distance from the centre. Computer models, calibrated with field data, predict mass concentrations of 100 kg km^{-2} in the inner GPGP compared to concentrations of only 0.1 - 0.01 kg km^{-2} beyond the outer boundary, and an exponential increase in plastic pollution of the GPGP, a much greater rate of increase than surrounding waters (Lebreton *et al.*, 2018).

The actions of atmospheric drag and wind waves on buoyant debris simulated the action of the wind by considering the displacement of particles as a fraction of the wind speed at 10 m above sea level, or the 'windage coefficient'. The best model representation utilised a windage coefficient of 0% and predicted the GPGP to be dominated by sea surface current driven particles, with particles experiencing atmospheric drag or wind-driven transport more likely to escape (Lebreton *et al.*, 2018). Plastic items captured in their trawls exhibited very little air draft when placed in seawater or appeared fully submerged in aerial photography. In this case, selective partitioning of synthetic debris occurs due to differences in wind-mediated transport of particles of differing buoyancy. Critchell and Lambrechts (2016) modelled the dispersal of plastic particles from coastal sources and found macroplastic debris can travel long distances in the direction of the wind, whereas suspended microplastic can disperse against the wind where currents are favourable. However, they used a simplistic parameter for "settling" as biofouling is difficult to model particularly for complex shapes. Ghost nets are large with a large surface area and they may be rapidly colonised by marine organisms, rather than floating proud of the surface they sink below the influence of rapid surface wind-mediated transport and may be preferentially retained due to their submersion. "Megaplastics" such as ghost nets are the main contributor of plastic mass in the GPGP, accounting for 46 % of total mass (Lebreton *et al.*, 2018). According to the model by Lebreton *et al* (2018), items with higher windage are transported further, are more likely to be beached on coastlines, and may escape ocean "garbage patches"; this may be the reason

why foam debris is almost non-existent in gyres, but occurs in high abundance on the coast (Eriksen *et al.*, 2013; Polasek *et al.*, 2017).

The transport of microplastics below the surface is a much slower process as they are submerged and dependent on currents rather than the wind (Obbard, 2015). Buoyant debris can be transported over long distances at sea relatively rapidly, as highlighted by the rapid appearance of buoyant debris from the 2011 Tohoku tsunami on the Western shores of the US (Lebreton and Borrero, 2013). Although winds may also influence the vertical distribution of plastic particles at the sea surface (Kukulka *et al.*, 2012). Multi-level net tows performed in the surface 5 m of the North Atlantic accumulation zone show that microplastic concentration decreases exponentially with increasing depth but the depth decay rate of plastic particles decreases with increasing Beaufort number, or wind speed (Reisser *et al.*, 2015). The effect of vertical mixing due to surface turbulence varies from particle to particle; Ballent *et al* (2012) assessed the submersion of particles using a variety of low density plastic items such as fragments, fibres, films, and foams, and observed that submersion was particularly affected by the density of particle followed by size and flatness. Films and filaments were drawn down at the lowest turbulence intensities of 2.5 cms^{-1}, the most resistant to surface turbulence were round LD pellets and foams. Therefore, the physical properties of a particle influencing its susceptibility to submersion under high turbulence are likely to impact time spent at the surface, and the effects of wind-mediated transport. Some particles may be drawn down below the surface to be transported by currents, whereas others spend more time at the surface and are transported by the wind (Critchell and Lambrechts, 2016).

1.2.3. Vertical movement of microplastics

Plastic pollution of the benthos is widespread, with densities of between 0 - 1835 pieces km^{-2} found on the seabed of the coastal seas of North West Europe (Maes *et al.*, 2018). These data were collected by bottom trawling with otter trawl nets (40 mm mesh gauge) designed for catching fish, so does not include micro-scale plastic. The estimated average global concentration of microplastic particles in benthic sediment is 1.5 - 6.7 particles kg^{-1} (as of 2010), and is predicted to

increase to between 73 and 373 particles kg^{-1} by the end of the century (Everaert *et al.*, 2018). Although a global analysis of deep sea sediments found concentrations as high as 1.4 - 40 microfibres per 50 mL sediment, exceeding both current and projected future concentrations (Woodall *et al.*, 2014). Despite continual growth in the annual production of plastic, Maes *et al* (2018) observed no statistically significant temporal trend in total macroplastic waste on the seafloor over a 25-year period (1992 - 2017), and with a constant influx from land and the surface this must indicate that this plastic is degraded or exported elsewhere (PlasticsEurope, 2018). Coastal currents are likely responsible for the transport of plastic waste into deeper waters and eventually off of continental shelves (Ballent *et al.*, 2013; Zhang, 2017). Unless resuspended, deep sea sediments are a likely final resting place for many sinking plastic particles which have been found in the deepest locations of the oceans, including in seawater at a depth of 2227 m in Rockall Trough (North Atlantic Ocean) and down to 3971 m in Monterey Canyon (California) (Schlining *et al.*, 2013; Woodall *et al.*, 2014; Courtene-Jones, Quinn, Gary, *et al.*, 2017). Benthic microplastic data are relatively scarce, so further field research is necessary to understand the true extent of microplastic pollution of the seafloor and the dynamics that result in transport of society's mismanaged plastic waste.

Previous research has almost exclusively focussed on the two extreme ends of the water column, making it apparent that plastic particles are mostly accumulated at the surface and the benthos, and until recently the microplastic load of intermediate depths of pelagic waters has been relatively unknown (e.g. Law *et al.*, 2014; Courtene-Jones, Quinn, Gary, *et al.*, 2017; Lebreton *et al.*, 2018). Plastic concentration data previously collected in the surface 5 m in the North Atlantic showed that microplastic particle abundance decays exponentially with increasing depth, however more recent research has revealed extensive plastic pollution of the epipelagic (~ 0 - 200 m depth) and the mesopelagic (~ 200 - 1000 m) zones (Reisser *et al.*, 2015; Choy *et al.*, 2019). Measurements of microplastic concentrations from the surface to 1000 m depth in Monterey Bay showed that the surface layer contained the lowest concentrations of microplastic (2.9 particles m^{-3}), which was also roughly equivalent to measurements at 1000 m, and microplastic abundance was greatest at intermediate depths, measured at 15 particles m^{-3} at 200 m.(Choy *et al.*, 2019).

Many factors influence the distribution of plastic particles in the water column including the physical properties of the particle. Particle density can have a significant impact on the position held in the water column. In a laboratory experiment using pre-production pellets collected from beaches in Los Angeles County, California, high density particles typically had greater settling velocities than low density particles, and transparent pellets seemed to split into two distinct groups with settling velocities of ~ 35 mms^{-1} and ~ 70 mms^{-1} (Ballent *et al.*, 2012). Particle shape also influenced suspension and rise velocity. However, the relationship between density and position in the water column is complex due to other factors, including interactions with marine organisms and organic matter.

Plastic particles form aggregates with organic matter and other synthetic particles that then fall as marine snow (Porter *et al.*, 2018; Zhao *et al.*, 2018). Marine aggregates collected in the field (Groton, Connecticut) contained 1290 ± 1510 plastic particles m^{-3}, or 1.3 ± 1.5 particles L^{-1}, which included polypropylene, polyester and cellulose acetate, which accounted for 44.7%, 21.2% and 11.8% of the total counts (Zhao *et al.*, 2018). Incorporation of plastic particles into artificial marine snows has been demonstrated in a laboratory setting, where aggregation increased the sinking rates of all tested synthetic plastic polymers, in particular polyethylene and polyamide by 818 and 916 m day^{-1}, respectively (Porter *et al.*, 2018).

Interactions with marine organisms can result in the transfer of microplastic through the water column, there is evidence of cycling of microplastics between the food-webs of intermediate pelagic waters, the sea surface and the deep sea (Choy *et al.*, 2019). Choy *et al.* (2019) showed that microplastics are ingested by particle-feeding organisms such as pelagic red crab *Pleuroncodes planipes* and giant larvaceans *Bathocordaeus spp*. Red crabs, which have both pelagic and benthic life stages, contained a median of 5 particles per individual and may act as a vector for microplastics throughout the water column. Giant larvaceans filter suspended and sinking particles from the water using excreted external mucus mesh filters which, upon becoming clogged with particles from feeding, are periodically discarded. These feeding apparatuses then sink and are known to transport significant amounts of carbon to the deep sea, but they also contain an

average of 10.7 ± 5.3 microplastic particles, demonstrating how microplastics may also be sequestered and rapidly transferred to the seafloor.

Colonisation by marine organisms, or biofouling, can impact the buoyancy of floating plastic particles which may result in movement of plastic particles away from the sea surface (Fazey and Ryan, 2016; Rummel *et al.*, 2017). Many species of marine organism utilise solid, floating substrates such as plastic fragments including red and green algae, hydroids, mussels, ascidians, bryozoans, barnacles, and other members of the plankton community; and plastic debris also hosts a distinct microbial community (Barnes and Milner, 2005; Fazey and Ryan, 2016; Miao *et al.*, 2019). While the composition of colonisers of synthetic polymers is unique, there is little variation in this community between polymer types (Kirstein *et al.*, 2018). The buoyancy of an item is a function of volume, and its susceptibility to fouling is dependent on its surface area, as an item decreases in size so its surface area and capacity to support biofouling increases relative to its volume (Ryan, 2015). Fazey and Ryan (2016) tested the hypothesis that smaller plastic items lose their buoyancy to fouling more quickly than large items using square LDPE and HDPE pieces of a range of sizes between 5 and 50 mm tethered beneath the water surface in a marina near Cape Town, South Africa. The majority of plastic pieces became negatively buoyant after 12 weeks and the smallest particles (5 x 5 x 0.1 mm (LDPE)) began to sink first. The study found a significant negative relationship between the time at which probability of sinking = 0.5 (P_{sink}) and the log of surface area:volume ratio. In addition, a significant positive relationship between P_{sink} and the log of the volume, which determines buoyancy. Larger particles are more resilient to sinking, a laboratory experiment on the impact of turbulence on surface mixing, showed only ~ 10 % of large fragments were submerged at even the highest turbulence dissipation rates whereas smaller fragments were affected at much lower turbulence (Ballent *et al.*, 2012).

The rate of colonisation and biofouling of plastic particles can be dependent on the habitat and season. For example, polystyrene and polyethylene microparticles incubated in estuarine and coastal waters are shown to have different sinking behaviours (Kaiser, Kowalski and Waniek, 2017). The sinking velocities of polystyrene particles were shown *in situ* to increase by 16 % in

estuarine water and by 81 % in marine water after a 6 week incubation period. A 14 week incubation period in estuarine water was insufficient to cause PE particles to sink, however particles began to sink in marine water after just 6 weeks. The colonisers differed between estuarine and coastal sites; coastal sites exhibited growth of small blue mussels *Mytilus edulis* and filamentous red algae, whereas estuarine sites showed a yellow-brown, fluffy growth which was not identified (Kaiser, Kowalski and Waniek, 2017). Surface longevity was influenced most strongly by macrofouling and development of a biofilm also increased the sinking velocity of negatively buoyant particles though alone did not cause particles to sink. In coastal water the rate of biofouling was seasonal, with short daylight hours and low temperatures reducing the amount of fouling (Kaiser, Kowalski and Waniek, 2017).

1.2.4. Coastline

Plastic is a pollutant of marine sediments in both benthic and intertidal zones (Turra *et al.*, 2014; Stolte *et al.*, 2015; Alomar, Estarellas and Deudero, 2016; Maes *et al.*, 2018). Microplastic has been reported in sediments in even the remotest regions, for example ~ 5 particles 10 ml^{-1} in sediments near Rothera Research Station, Antarctica (Reed *et al.*, 2018). There is notable focus on plastic pollution of the coastline as both an aesthetic and environmental issue. A 10-year assessment of “beach clean” data from UK beaches shows almost 2.4 million waste items were collected from 1402 km of surveyed coastline, of which ~ 76 % were plastic (Nelms *et al.*, 2017).

Plastic debris along the coastline is a combination of oceanic plastic from distant sources, items lost at sea, and local land-based sources (Lo *et al.*, 2018; Yu *et al.*, 2018). In the review of UK citizen science data noted above, of items collected that could be attributed to an origin, 42 % derived from land-based sources; represented most commonly by discarded food packaging from littering and mismanaged waste, and sanitary items from sewage. Public “beach cleans” typically do not include plastic at the micro-scale, usually going down to the scale of nurdles or “mermaids tears”. These are plastic pre-production pellets that are roughly at the upper size limit used by most studies for microplastic at 5 mm,

which could be more suitably classed as meso-plastic (Hermabessiere *et al.*, 2017). However, based on current and projected values of plastic production and losses to the oceans, average global concentrations of 32 - 144 microplastic particles kg^{-1} of dry sediment are estimated in beach deposition zones (Everaert *et al.*, 2018). By the year 2100, this value is predicted to increase to 1580 - 8050 particles kg^{-1}, although factors resulting in unequal distribution and accumulation zones can produce areas that already exceed the minimum “safe” concentration of 540 particles kg^{-1} at which organism effects may occur based on species sensitivity data (Moreira *et al.*, 2016; Everaert *et al.*, 2018).

At the coastline a variety of physical dynamics may influence the transport of microplastic particles (Zhang, 2017). Although the most critical factors determining the fate of plastic particles are the source, the physical properties of the particle, and the interplay between wind, surface currents, and the local topography. Critchell and Lambrechts (2016) used modelling to run simulations of macro- and micro-plastic dispersal in the Whitsunday region of the Great Barrier Reef, incorporating beaching, re-floating, settling to the seafloor, and degradation of particles to microplastic as possible physical processes. They found that the source location was the most important parameter, in a rugged topography a difference of only a few kilometres made a very large difference to the fate of the plastic particles and in particular had a major influence on which beaches the plastics accumulated on and to the location and size of the plumes of suspended plastics. The properties of the particle also played a role in transport dynamics around the coast as they also found particle size to have an impact on its distribution, macroplastic particles were driven far from the seeding location in the direction of the wind, whereas microplastics in suspension could disperse against the wind direction where currents were favourable.

Wind direction and currents can have a major influence on the composition and abundance of beached plastic on the coast. Atwood *et al.* (2019) used a hydrodynamic particle tracking model to simulate particle dispersal from rivers in Northern Italy and found beaching rates of particles from rivers were highly variable, from < 10 % up to 94 %, and of all simulated particles released only 18% were beached, the rest being released to the open Adriatic Sea. Beached microplastic abundance was far more strongly influenced by local surface

currents than the total abundance in river water. Satellite remote sensing of river plume form showed that changes in wind regimes and freshwater discharge of a river can alter the shape and direction of the river plume and therefore the transport of particles and likelihood of deposition on the coast (Atwood *et al.*, 2019). Microplastic particles collected on the Chennai coast are influenced by seasonal changes in surface currents and winds, anti-cyclonic surface currents caused by south-westerly winds induce particle transport away from the coast but a change in direction of wind and current during the onset of NE monsoon winds later in the year changes the direction of particle transport towards the coast, allowing the beaching of particles (Veerasingam *et al.,* 2016).

The properties of particles and physical conditions around the coast may result in "coastal trapping" of plastic particles. Globally, the average microplastic particle concentration in intertidal zones (32 - 144 particles kg^{-1}) is estimated to be about twenty-fold higher than deep sea sediments (1.5 - 6.7 particles kg^{-1}) (Everaert *et al.*, 2018). These processes are concerning as it would mean plastic accumulation is occurring in a region coinciding with great ecological and economic importance (Seitz *et al.*, 2014). Isobe *et al.* (2014) found evidence for selective transport of certain plastic particles in coastal waters. Key in their findings was that the size and quantity of mesoplastics gradually increased close to the coast regardless of the proximity to river mouths. They theorise there are selective methods for "near-shore trapping" of mesoplastics as offshore no plastic particles greater than a few millimetres were found, whereas plastic particles < 1 mm were numerous offshore. This has also has been observed in the Mediterranean sea where plastic concentrations are highest in regions distant from land as well as in the first kilometre adjacent to the land (Pedrotti *et al.*, 2016). A combination of buoyancy force and stokes drift has been suggested as the physical dynamics responsible for selective transport of mesoplastics. In the turbid upper layer of the water fragments are mixed vigorously, particles then move upward due to their buoyancy, as larger particles have a greater upward terminal velocity they rise more quickly. Wind waves cause stokes drift of particles, which is greater in surface layers than in the deeper layers, therefore resulting in transport of the larger mesoplastics particles to the shore at a greater rate than microplastics (Isobe *et al.*, 2014).

Fragmentation and decay of plastic debris is particularly high for beached particles on the coast as particles experience greater temperatures and UV exposure than at sea, which increases the effects of photo- and thermo-oxidative degradation in addition to the physical forces of wave action (Andrady, 2011). The change in size of the particle may ultimately lead to the release from the selective retention processes acting upon the particle at the meso-scale and therefore allow dispersal of the micro-particle away from the shore.

Ballent *et al* (2012) used an experimental 20 cm erosion microcosm to determine the flow velocities at which bedload, resuspension and deposition of three categories of plastic pellets occur. The study found a differing capacity for resuspension between pellet types, at the highest possible shear stress in the chamber (~ 0.2 N m^{-2}) nearly all black pellets were in suspension however the majority of the opaque/transparent pellets were not significantly suspended, showing a greater resistance to erosion from the sediment. Using their results for HD black pellets the study also used hydrodynamic modelling to generate benthic transport predictions. Simulated pellets originating at depths between 59 and 2657 metres showed that the pellets travelled an average of 0.1 km but showed little displacement from their origin point, only 0.04 km, describing an oscillating movement. Tidal forces near the shore transported particles readily but dispersed them very slowly, only 0.05 - 2.6 % of pellets escaped from their original location over a 56 day period. This limited capacity for dispersal in some particles might be another factor which results in the “trapping” of particles at the coastline once they have deposited in sediment.

1.3. Factors affecting uptake in organisms

Physical factors influence the distribution of microplastic particles in the marine environment and ultimately the particles encountered by marine organisms (particularly for sedentary or sessile organisms). Here I will discuss some examples of how the concentration of exposure, physiology, and behaviour influence uptake of plastic particles. The ingestion of certain polymers and particle shapes, and relative amount of ingestion is likely determined by their

availability in the surrounding environment and the feeding mode of an organism (Galloway, Cole and Lewis, 2017; Pazos *et al.*, 2017; McNeish *et al.*, 2018; Qu *et al.*, 2018). However, the variability of marine fauna in form, habitat, and feeding strategy in addition to the complexity of synthetic polymer debris in the marine environment means these relationships are likely relatively unique to each species and location. It is known that uptake of plastic in marine organisms is widespread in numerous marine taxa including birds, mammals, fish, and invertebrates (Gall and Thompson, 2015). Mortality due to macroplastic ingestion has been observed in marine mega-fauna, but the direct impact of microplastic ingestion less well understood (Jacobsen, Massey and Gulland, 2010; Wilcox *et al.*, 2018).

The amount and type of microplastic ingested likely determines the level of biological effect. A recent synthesis of ecotoxicological endpoints for threshold effect concentrations showed the lowest observed effect concentrations for algae, invertebrates, and fish (for particles > 10 µm) at ~ 10^4 to 10^8 particles/L (Burns and Boxall, 2018). Another meta-analysis of effect data provides a much lower concentration of 6650 particles m^{-3} of water as a lowest "safe" concentration before adverse effects occur (Everaert *et al.*, 2018). The true ecological impact of microplastic particle ingestion remains difficult to assess. Impacts at the cellular level may be felt throughout the levels of biological organisation; impacting tissues, then organs, thus altering the physiology and behaviour of the individual and therefore the population, eventually to ecosystem-scale effects (Galloway, Cole and Lewis, 2017). However, to begin to understand the biological effects of microplastic we must understand what particles are actually ingested by a particular organism. Different shapes and sizes of microplastics may have different uptake dynamics and toxicological endpoints. Therefore, understanding the factors that influence uptake are key to understanding potential impacts.

Measuring the amount of plastic exposure for fish is challenging as they are highly mobile, and the degree of exposure could change rapidly over time. An assessment of microplastic ingested by mesopelagic fishes and the microplastic concentration of the surrounding sea water found no correlation (Lusher *et al.*, 2016). Although, in Río de la Plata estuary, Argentina, examination of the microplastic content of 11 coastal freshwater fish species showed microplastic

content of fish was significantly greater in fish sampled close to points of sewage discharge (Pazos *et al.*, 2017). Uptake in this case was not influenced by species or group feeding traits, indicating that in some cases the role of environmental availability may be of greater influence on uptake. The majority of plastic particles are destined to sink and accumulate at the sea floor, and in theory the global average concentration in surface waters (0.2 - 0.9 particles m^{-3}) is lower than in benthic sediments (1.5 – 6.7 particles kg^{-1}) (Everaert *et al.*, 2018). So, we might expect uptake to be greater in benthic species compared to pelagic due to greater exposure. However, evidence of microplastic ingestion by fish species has revealed no clear overall pattern, or even evidence to the contrary. In the North and Baltic seas, the prevalence of plastic ingestion in pelagic fish species (herring and mackerel) was greater than in demersal fish (cod, dab, and flounder), 10.7 % versus 3.4 %, respectively (Rummel *et al.*, 2016). A comparison of the gut microplastic content of five pelagic and five demersal fish species from the English Channel found no significant difference between pelagic or demersal species (Lusher, McHugh and Thompson, 2013). This suggests that regardless of feeding habits and location, low levels of ingestion of microplastic particles is common amongst pelagic and demersal fishes, which on average contained 1.9 (± 0.01) particles per individual with little variability between species.

Once organisms encounter plastic particles in their immediate environment other factors relating to physiology, behaviour, and feeding ecology of a species are likely to further influence likelihood of ingestion. In a study of 11 different taxa of freshwater fishes in tributaries of Lake Michigan, USA, the functional feeding group had a significant effect on microplastic content of the gut (McNeish *et al.*, 2018). Between the three rivers sampled there was no significant difference in particle abundance in the fish despite different abundances in each river, however, there were significant differences across fish species and functional feeding groups. Across all sites zoobenthivores, such as round goby (*Neogobius melanostomus*), contained significantly more microplastic particles than detritivores, such as fathead minnow (*Pimephales promelas*) and white sucker (*Catostomus commersonii*); and in the Milwaukee river zoobenthivores contained more plastic than omnivores. However the functional feeding group can’t exclusively govern uptake as differences in plastic ingestion have been observed between species of a similar functional feeding group (Halstead *et al.*, 2018).

Yellowfin bream (*Acanthopagrus australis*), sea mullet (*Mugil cephalus*) and silverbiddy (*Gerres subfasciatus*) are all benthic-foragers. However, individuals collected in an urbanised estuary near Sydney harbour, Australia, showed that sea mullet and yellowfin bream both ingested significantly greater amounts of plastic particles than silverbiddy (Halstead *et al.*, 2018). Therefore, other organism traits may also have a role in the chance of uptake of plastic particles, rather than feeding habits alone, though observed patterns in uptake are mixed or unapparent. A study of six families of mesopelagic fish in the North East Atlantic found no difference in the number of plastic items in the digestive tract between species, time of day, or diel vertical migration patterns (Lusher *et al.*, 2016). And this is not exclusive to fish, in tropical sea birds in the Hawaiian Islands examination of the gut content of four foraging guilds including albatrosses, nocturnal-foraging petrels, plunge-divers, tuna-birds and terns found no significant difference in plastic prevalence across foraging guilds suggesting that all feeding behaviours in this instance are equally as likely to result in some level of ingestion of marine plastic particles (Rapp *et al.*, 2017). Factors influencing plastic uptake are difficult to predict potentially due to the complex nature of the pollutant, and the likelihood of plastic ingestion may still be somewhat dependent on chance and the choices of the individual organism.

The factors influencing uptake in marine turtles are also complex. On the one hand, the relative exposure due to geographic differences has been shown to be a more significant factor influencing ingestion of plastic debris than species feeding traits (Duncan *et al.*, 2019). Here, all turtle species' showed evidence of plastic ingestion despite occupying different trophic niches and utilising different feeding strategies, indicating multiple uptake pathways. Turtles of the same species had a higher incidence of plastic in the Mediterranean than in the Pacific or Atlantic; including loggerhead turtles (*Caretta caretta*) and green turtles (*Chelonia mydas*) which were present at all three studied ocean basins (Duncan *et al.*, 2019). However, on the other hand another review of debris ingestion in turtles suggests that there are species specific risks of ingestion and the probability of ingestion is not linked to modelled debris densities ("debris" is mostly all plastic though this also includes some non-plastic items) (Schuyler *et al.*, 2014). Leatherback and green turtles were significantly more likely to ingest

debris than Kemp's Ridley or loggerhead turtles, and carnivorous species were less likely to ingest debris than herbivores, gelatinovores, or omnivores. Additionally, life-stage may also play a role in uptake as smaller oceanic-stage turtles are more likely to ingest debris than coastal foragers (Schuyler *et al.*, 2014). Young turtles are typically opportunistic feeders whereas adults have a more specialised diet, this might make them more likely to ingest floating plastic particles. Duncan *et al* (2019) found plastic microbeads exclusively in post-hatchling turtles in the Pacific suggesting that these young turtles may be exposed to different types of microplastic particles due to their epipelagic lifestyle.

In the subtropical convergence zone to the North-East of Hawai'i, the Ekman transport and accumulation of plastic particles at the surface coincides with high biomass of natural prey items, and therefore could be an area of increased likelihood of uptake (Lebreton *et al.*, 2018). Evidence for this can be seen in Black-footed albatross (*Phoebastria nigripes*) chicks in which micro- and mesoplastics particle ingestion is pervasive (Kure Atoll, Hawai'i). Satellite tracking of the foraging trips of chick-provisioning adult birds revealed that they spend most of their time feeding within the latitudinal band influenced by the subtropical convergence (Hyrenbach *et al.*, 2017). The Laysan albatross (*Phoebastria immutabilis*) also feeds throughout the North Pacific, and there is also a significant impact of foraging distribution on plastic items fed to chicks (Young *et al.*, 2009). Tracking data shows that adult birds from the two study sites, Oahu Island and Kure Atoll, have distinct core feeding grounds during the early breeding season. The Kure Atoll population's feeding ground includes a greater overlap with the GPGP, and chicks from this group are fed up to ten times more plastic than those from Oahu despite the regurgitated boluses of indigestible material containing a similar amount of natural food.

Similar trends are reported in North Sea fulmar (*Fulmarus glacialis*), a species well established as a bioindicator of marine plastic pollution (Franeker, Rebolledo and Meijboom, 2017). The prevalence of industrial plastic pellets in fulmar and the North Atlantic subtropical gyre over time are both very similar, both decreasing by ~ 75 % since the 1980's, although there is no obvious change in either for "user plastics" (Franeker and Lavender, 2015). Lavers and Bond (2016) suggest that foraging location and differential provisioning for fledglings can

impact exposure to plastic particles. Among shearwaters, adults often make long offshore trips to feed themselves but forage closer to the coast for chick-provisioning, the coast being a region of high plastic accumulation due to “coastal trapping” processes (Lavers and Bond, 2016; Pedrotti *et al.*, 2016). Likewise, black-footed albatross chicks contain significantly more plastic particles than adults, although for Laysan albatross there is no difference between chick and adult (Hyrenbach *et al.*, 2017; Rapp *et al.*, 2017).

Interactions between plastic and marine life will likely be common close to the coast where both microplastic concentrations and biological activity are relatively high (Seitz *et al.*, 2014). In the open ocean interactions between plankton and microplastics may not be as common. One concern of microplastic pollution is that microplastic ingestion by zooplankton, which has been demonstrated experimentally in mysid shrimps, copepods, decapod larvae, cladocerans, rotifers, polychaete larvae and ciliates, could result in small microplastic particles becoming available to larger zooplanktivores by trophic transfer (Cole *et al.*, 2013; Farrell and Nelson, 2013; Setälä, Fleming-lehtinen and Lehtiniemi, 2014; Chae *et al.*, 2018). However, Clark *et al* (2016) reports a spatial mismatch in the distribution of microplastic particles (< 200 µm) and ocean chlorophyll distribution, a proxy for ocean primary production. Primary production is relatively low in the subtropical gyres, regions of downwelling which depress the thermocline and limits surface nutrients, relative to the subpolar gyres which are regions of upwelling. Therefore, although subtropical gyres are well known for accumulation of plastic, interactions with planktonic organisms there may be infrequent due to relatively low levels of biological activity.

Biological impacts may be more common where points of accumulation overlap with regions of ecological importance. Sea-ice in particular is a highly productive habitat for marine algae and invertebrates, acting as a feeding ground for amphipods and copepods, and nursery habitat for juvenile krill *Euphausia superba* a keystone species in the Antarctic ecosystem (Poltermann, 2001; Arrigo and Thomas, 2004). This coincides with reported regions of accumulated plastic debris, as the Arctic oceans are emerging as a region of extensive plastic pollution (Lusher *et al.*, 2015; Obbard, 2015; Cózar *et al.*, 2017; Tekman, Krumpen and Bergmann, 2017). Even more concerning is that sea ice has

recently been described as a temporary sink for marine microplastic particles, containing highs of 1.2 (± 1.4) x 10^7 particles m^{-3} in pack ice in the Fram Strait (Peeken *et al.*, 2018). These particles are sequestered from the surrounding water in high concentrations as the ice forms and released as the ice melts, and this results in large concentrations of microplastic particles occupying an important Arctic marine habitat, the interface between sea-ice and water. Therefore, the likelihood of uptake in small marine fauna occupying Arctic sea-ice may be high. Further understanding partitioning of plastic particles in the marine environment may provide insight into regions and organisms at risk, and the likely ecological impacts of exposure to microplastic particles.

Uptake and retention by an organism can also be dependent on the physical characteristics of the particle. For example, in the Norway lobster *Nephrops norvegicus*, the physiology of the gut appears to be prone to entanglement by filament/line type plastic debris, 62 % of studied individuals collected in the Clyde Sea contained compacted balls of filamentous plastic (Murray and Cowie, 2011). As a predator/scavenger the gut system of this crustacean includes a complex “gastric mill” system and filter apparatus designed for grinding soft tissue and crustacean carapaces into fine particles before entering the stomach. This system is not designed to break down resilient fibres and therefore they may accumulate in the gut.

The colour and shape of the particle might also influence uptake dependent on feeding behaviour of the organism. There is evidence of selectivity based on colour/shape of particles in flesh-footed shearwaters (*Ardenna carneipes*) in Australia (Lavers and Bond, 2016). Adults ingested black and white particles at a greater frequency than available in the environment and had a mild preference for delivering white particles to their chicks but a strong aversion to yellow/orange, purple, and red/pink items. Selection was shown to be strong at Lord Howe Island (LHI) but not at King George Sound (KGS), the authors suggest the dominant food species of each bird population as a possible explanation for the differing colour preferences. Data from the LHI population suggests their chicks are mainly provisioned with squid which may explain the preference for white plastic, whereas data for the KGS group suggests a preference for pilchards *Sardinops sagax neopilchardus* and lanternfish *Myctophidae* which may explain the

preference for darker coloured plastic (Lavers and Bond, 2016). Additionally, the age class of the individual also impacted the abundance and types of plastic ingested, fledglings contained more plastic items than adults, and adults frequently ingested small rope fragments (34.8%) which were absent from fledglings. Hyrenbach *et al* (2017) notes that flying-fish egg masses, a component of black-footed albatross diet, are often attached to floating synthetic foam which may be ingested accidentally with natural food; foam was major component of chick boluses and stomachs.

1.3.1. Ingestion in filter-feeding bivalves

In chapter 3 I will further explore the uptake of microplastic particles by organisms in comparison to the particles available in their surrounding environment. To achieve this, I will focus on blue mussels *Mytilus edulis*, as they are common on rocky shores, ecologically and economically important, filter-feeders which play a significant role in benthic-pelagic coupling, and are previously well established as bioindicators of marine pollutants (Nielsen and Maar, 2007; Beyer *et al.*, 2017). Therefore, in this section I will review some of the current knowledge of microplastic particle uptake in *M. edulis* and other bivalve species.

Microplastic uptake in bivalves is relatively well established, reported globally in wild and cultured individuals (Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe *et al.*, 2015; J. Li *et al.*, 2018; Phuong *et al.*, 2018; Cho *et al.*, 2019). Many bivalves inhabit the coastline where microplastic concentrations are high, and positive correlations between environmental concentrations of microplastic and the number of particles ingested have already been reported (Bråte *et al.*, 2018; Qu *et al.*, 2018). Bivalves process large volumes of water and efficiently ingest small particles, therefore are often considered one of the most likely groups of organisms to ingest microplastic particles (Ward and Shumway, 2004; Riisgård, Larsen and Pleissner, 2014). In a mesocosm experiment, bivalves (*Mytilus trossolus* and *Macoma baltica*) have been shown to have significantly greater uptake of 10 µm polystyrene beads compared to other marine invertebrates including amphipods, mysid shrimps, and polychaetes (Setälä, Norkko and Lehtiniemi., 2016). Also, the prevalence (% of organisms

containing particles) of uptake between species was different; at the lowest exposure levels 90 % of bivalves contained microplastic particles compared to just 0 – 20 % of individuals in other taxa. In this case feeding mode clearly plays a role in the relative amount of uptake of microplastic particles.

However, another study compared microplastic ingestion in 10 marine species including bivalves, brittlestars, heart urchins, polychaetes, shrimps and fish, and found filter-feeders to contain significantly fewer particles per individual than predators (Bour *et al.*, 2018). The number of polyethylene particles ingested was significantly higher in predators compared to deposit- and filter-feeders, in tertiary consumers compared to primary and secondary consumers, and in demersal and bentho-pelagic species compared to benthic.

Feeding behaviour and trophic level influence the likelihood of ingestion, and as with other organisms the uptake of particles is likely determined by a combination of environmental availability, behaviour and the physiology. Complex feeding structures can result in entanglement and adherence of particles to tissues, but filter-feeding is not entirely passive and particle selection processes play a role in ingestion allowing selective rejection of larger particles as pseudo-faeces, possibly based on the relative organic content of the particle (Defossez and Hawkins, 1997; Kolandhasamy *et al.*, 2018). Experimental exposure of *M. edulis* to microplastic fibres (30 fibres mL^{-1}), showed that 71 % of particles were rejected as pseudo-faeces, compared to 9 % ingested (Woods *et al.*, 2018). Typically, fibres constitute the majority of particles ingested by bivalves and excluding cellulosic materials, PE, PP, PET, PS, and PA are the most commonly identified polymers in mussels (*Table 1.1.*). Between wild and cultured mussels, quantitative and qualitative differences in the particles ingested have been observed (Mathalon and Hill, 2014; J. Li *et al.*, 2018). This importance of local sources of microplastic in determining uptake may be highlighted the observation by Mathalon and Hill (2014) that the most common polymer in wild individuals is polyester but in cultured is polypropylene, likely from the ropes used as a substrate.

Filter-feeding bivalves are a class containing key species' of interest in terms of potential pathways of microplastic particles into the marine food chain and human

diet (Cho *et al.*, 2019). Bivalves can be exceedingly numerous in some habitats and form a part of the diet of many coastal species in the water and on land, and trophic transfer of micro- and non-plastic particles has previously been demonstrated experimentally (Bayne, 1976; Farrell and Nelson, 2013; Chae *et al.*, 2018). Evidence of trophic transfer from macroinvertebrates to shorebirds in estuary habitat is demonstrated by greater similarities between morphologies of microfibres in shorebird gizzards/faeces and macroinvertebrates (polychaetes and bivalves) compared to sediment, suggesting the fibres are sourced from prey (Lourenço *et al.*, 2017). Mussels are known to absorb and accumulate other anthropogenic pollutants such as metals, PAHs, and PCBs, efficiently capturing substances from the water and making them available to other organisms, and similarly mussels contain greater concentrations of microplastic by weight than surrounding seawater (Beyer *et al.*, 2017; Karlsson *et al.*, 2017). Microplastic particles may even exacerbate the transfer of pollutants such as PAH to exposed mussels (Avio *et al.*, 2015). However, accumulation of microplastic in mussels is not indefinite as particles are transient in the digestive tract and egested in faeces, though this may not be the case for small tissue bound particles (von Moos, Burkhardt-Holm and Koehler, 2012; Woods *et al.*, 2018). The contribution of microplastics to other organisms through trophic transfer might not be any more significant than direct ingestion, but they might make the smallest microplastics bioavailable to larger organisms.

Species	Location	Quantity	Dominant shape	Dominant polymers*	Particle size**	Reference
Mytilus edulis	UK	0.7 - 2.9 items g^{-1}; 1.1 - 6.4 items $individual^{-1}$	Fibre (~50 - 90%)	PE, PP	73 µm - 4.7 mm R	Li *et al.* (2018)
M. edulis, Perna viridis	China	1.52 - 5.36 items g^{-1}; 0.77 - 8.22 items $individual^{-1}$	Fibre (86%)	PET (77%)	0.25 - 1 mm C	Qu *et al.* (2018)
M. edulis	South Korea, fish market	0 - 0.35 items g^{-1}; 0 - 2.4 items $individual^{-1}$	Fragment	PS (33%), PP (~27%)	100 - 200 µm C	Cho *et al.* (2019)
M. edulis	France	0.23 ± 0.2 items g^{-1}; 0.6 ± 0.56 items $individual^{-1}$	Fragment	PP (47%), PE (38%)	50 - 100 µm C	Phuong *et al.* (2018)
Mytilus spp.	Norway	0.97 ± 2.61 items g^{-1}; 1.5 ± 2.3 items $individual^{-1}$	Fibre (83%)	"Parking lot tar" & EVA foam (18.7%)	70 - 3870 µm (av. 770 µm) R	Bråte *et al.* (2018)
M. edulis	UK	1.05 (±0.66) - 4.44 (±3.03) items g^{-1}	Fibres (86%)	Polyamide (72%)	0.2 - 10.7 mm (av. 1.22 mm) R	Courtene-Jones, Quinn, Murphy, *et al.* (2017)
M. galloprovincialis	Greece	5.3 ± 0.5 items g^{-1}; 0.8 ± 0.2 items $individual^{-1}$	Fragments (77.8%)	PE (75%)	0.1 - 0.5 mm (52.6%) C	Digka *et al.* (2018)
M. galloprovincialis	China, fish market	~ 2.2 (±1.1) items g^{-1}; ~ 4 (±3) items $individual^{-1}$	Fibre (67%)	PE, PET	5 - 250 µm (~ 60%) C	Li *et al.* (2015)
M. galloprovincialis	Italy	4.4 - 11.4 items g^{-1}; 3 - 12.4 items $individual^{-1}$	Fibre (100%)	N/A	1.7 (± 0.37) - 1.9 (± 0.75) mm R	Renzi, Guerranti and Bla, (2018)
M. edulis	Netherlands	13.2 items g^{-1}	Fibre	N/A	10 - 300 µm (50 - 82 %) C	Leslie *et al.* (2017)
M. edulis	Netherlands	6 - 107 items g^{-1} (d.w.)	Fibre (50%)	N/A	30 - 2000 µm (av. 200 µm) R	Karlsson *et al.* (2017)
Mytilus spp.	Belgium	0.26 - 0.51 items g^{-1}	Fibre	N/A	1000 - 1500 µm C	De Witte *et al.* (2014)

*excluding cellulose
** R = Range; C = Common size range

Table 1.1. Properties of microplastic particles found in *Mytilus spp.* by recent studies, including the average quantities and the most common morphologies and constituent polymers.

1.4. Conclusion

Local topography, hydrography, sources of pollution, and interactions with marine life are among the numerous factors that influence the distribution of microplastic particles in our oceans. Due to these factors and characteristics of microplastic particles, regions of accumulation are beginning to emerge which are potential areas of elevated risk of uptake for organisms. However, particle concentration is only one aspect in plastic uptake and species-specific physiologies and behaviours may determine the possibility and likelihood of plastic particle ingestion. Identification of at-risk areas, or at-risk species, is paramount to understanding the implications of the mismanagement of our synthetic waste and for targeting efforts to reduce the extent of the complex pollutant known as plastic.

In this thesis I use environmental sampling campaigns of two contrasting marine habitats to investigate the evidence of plastic partitioning in the marine environment. Chapter 2 investigates the microplastic particle content of two different depths in an Arctic fjord to attempt to assess differences in the plastic pollution of different water bodies, local and Atlantic. Atlantic water is hypothesized as a vector for plastic waste carried to the Arctic. As in a typical fjord system this water enters fjord at depth, below local water due to stratification processes. I test the hypothesis that:

H_1: Atlantic Water in deeper regions of an Arctic fjord system contains greater concentrations of microplastic particles due to the origin of this water mass, with different physical characteristics, compared to plastic particles in local Arctic surface waters.

Chapter 3 then investigates the microplastic content of a common organism of rocky shore habitats, blue mussels *Mytilus edulis*, from the coasts of Devon and Cornwall. As a filter-feeder they are likely susceptible to uptake of waterborne plastic and have previously been used as bioindicators of other marine pollutants. I compare observations of microplastic particles in their surrounding environment, in surface water and intertidal sediment, to assess which particles are bioavailable to this

organism and whether uptake is representative of environmental availability or if selection processes occur. This work tests the hypothesis:

H_2: Microplastic particles partition differently according to physical characteristics of particles, such as size, shape, or polymer type, in different marine compartments (water, sediment, and mussels *Mytilus edulis*) of rocky shore habitats in South-West England.

Chapter 2

Microplastic pollution in Arctic waters: Physical characteristics of microplastic particles in surface and deep waters of Kongsfjorden, Svalbard.

2.1. Introduction

Plastic pollution of the world's oceans is a well-known environmental issue. The continuing rise in the annual production of plastic polymers and mismanagement of plastic waste has led to an estimated 4.8 - 12.7 million tonnes of plastic entering the marine environment each year (Jambeck *et al.*, 2015). Global marine plastic pollution is predicted only to increase under current future scenarios, the total mass of floating microplastics is predicted to increase to between 2.5 x 10^7 and 1.3 x 10^8 tonnes (best and worst case scenario, respectively) by the year 2100 (Everaert *et al.*, 2018). Recently public awareness of microplastic and sources of marine plastic pollution has grown, however our understanding of the ultimate fate of plastic in the marine environment is incomplete. Floating plastic debris in the open ocean, estimated at between 93 and 236 thousand metric tonnes from surface monitoring data, is only a small fraction of the plastic estimated to enter the oceans each year, leaving the majority of all plastic thought to enter the marine environment unaccounted for (Cozar *et al.*, 2014; Van Sebille *et al.*, 2015; Geyer, Jambeck and Law, 2017). The distribution of plastic debris in the marine environment is uneven, surface ocean plastic debris is transported by oceanic currents which has resulted in accumulation zones, notably these include the sub-tropical oceanic gyres which are well established as regions of plastic accumulation (Eriksen *et al.*, 2013; Cozar *et al.*, 2014; Lebreton *et al.*, 2018). More recently, evidence is emerging that suggests the Arctic Ocean is another region of accumulation of marine plastic (Obbard *et al.*, 2014; Lusher *et al.*, 2015; Cózar *et al.*, 2017; Kühn *et al.*, 2018; Peeken *et al.*, 2018).

The Arctic is an environment that is often considered to be remote and pristine, however it is at the forefront of environmental change (Fabry *et al.*, 2009). The region is experiencing disproportionate rates of climate warming and ocean acidification as a result of anthropogenic CO_2 emissions, and a major decline in the extent and longevity

of seasonal sea ice (Maslanik *et al.*, 2007; Steinacher, Joos and Fr, 2009; Screen and Simmonds, 2010; Lindsay and Schweiger, 2015). Human activities are poised to increase with the retreat of sea ice, for example open ice-free seas allow direct transport of goods via new shipping lanes, and further exploitation of undersea gas and oil, and alternative and shifting fish stocks that become commercially viable (Mcbride *et al.*, 2014; Pierre and Olivier, 2015; Harsem, Eide and Heen, 2017). Plastic pollution is just one component of the human influence on this region, and is rapidly emerging as another potential threat to the ecologically diverse Arctic marine ecosystem (Hop *et al.*, 2002, 2006).

The Arctic has recently been suggested as a potential 6th microplastic accumulation zone (Bergmann, Wirzberger, *et al.*, 2017), with microplastics having been recorded in Arctic surface and subsurface waters (Lusher *et al.*, 2015; Kanhai *et al.*, 2018), sediments (Bergmann, Wirzberger, *et al.*, 2017; Kanhai *et al.*, 2019), sea ice (Obbard *et al.*, 2014; Peeken *et al.*, 2018), benthic organisms (Fang *et al.*, 2018), fish (Kühn *et al.*, 2018), and seabirds (Amélineau *et al.*, 2016) across the Arctic Ocean. For example, in Arctic waters south and south-west of Svalbard, microplastic particles have been reported in surface (top 16 cm) and sub-surface (6 m) samples (Lusher *et al.*, 2015). The average concentration in surface water and in sub-surface water was 0.34 and 2.68 particles per m^3, respectively. Almost all of these particles were fibres (95 %), and mostly were made of cellulose (30 %), polyester (15 %), and polyamide (15 %). Microplastics are also present in the Arctic Pacific, including the Bering and Chuckchi seas, with a mean abundance of 0.13 particles per m^3 (Mu *et al.*, 2019). These samples were also predominantly fibres (96 %), and constituted mostly PET (68 %), PP (11%), and PA (7 %). Accumulation of plastic debris is also present in Arctic deep sea sediments from the HAUSGARTEN observatory, where concentrations of 42 - 6595 particles kg^{-1} are reported, and were mostly very small particles (< 25 µm) of chlorinated PE (38 %), PA (22 %), and PP (16 %) (Bergmann, Wirzberger, *et al.*, 2017).

Two independent studies both reported the presence of high concentrations of microplastic particles in Arctic sea ice cores (Obbard *et al.*, 2014; Peeken *et al.*, 2018). Obbard *et al.* (2014) found 38 - 234 particles m^{-3}, and reports this to be mostly rayon (54 %), polyester (21 %), and polyamide/nylon (16 %). However, Peeken *et al.* (2018)

reports much greater concentrations, from 1.1 (± 0.8) × 10^6 to 1.2 (± 1.4) × 10^7 particles m^{-3}, the highest found in ice cores taken from the pack ice of the Fram Strait. These were mostly all very small particles, 67 % were of the lowest detectable size limit of 11 µm, and 48 % were polyethylene. These exist at concentrations several orders of magnitude higher than in waters that are considered highly contaminated, such as the Pacific gyres (Eriksen *et al.*, 2014; Law *et al.*, 2014; Lebreton *et al.*, 2018; Peeken *et al.*, 2018). Between 2002 and 2014, a strong increase in marine litter has been observed at two stations of the HAUSGARTEN observatory at a depth of 2500 m in eastern Fram Strait (Tekman, Krumpen and Bergmann, 2017). Almost half was plastic (47 %), however litter density positively correlated with the amount of shipping activity suggesting that a proportion of the litter originates from local sources rather than long range transport for larger items. However the ratio of marine surface plastic (g) to coastal inhabitant is much higher in the Arctic than in any other ocean basin, suggesting this plastic is derived from distant sources rather than locally (Cózar *et al.*, 2017).

Interactions between microplastic and organisms may be more frequent in the Arctic compared to subtropical gyres as organisms and plastic co-occur in high numbers in these seasonally highly productive waters. Plastic is abundant in surface waters of the subtropical gyres however the relative amount of biota (using sea surface chlorophyll as a proxy for oceanic primary production) is low (Clark *et al.*, 2016). The Arctic ocean ecosystem is also diverse, supporting abundant marine mammal and seabird communities (Hop *et al.*, 2002). For which there exists photographic evidence of the negative impacts of entanglement on the northern coastline of the Spitsbergen archipelago (Bergmann, Lutz, *et al.*, 2017). Kongsfjorden, Svalbard hosts seals, walrus and whales which typically peak in the summer months due to migratory behaviours, and plastic ingestion has been observed in similar marine mammal species including harbour seal, common dolphin, and whales (True's beaked, and humpback) through observations of stranded and bycaught individuals (Lusher *et al.*, 2018). Plastic ingestion has also been observed in juvenile polar cod *Boreogadus saida*, in Kongsfjorden and north of Spitsbergen over the Svalbard shelf; this is a key species in the Arctic ecosystem, although in this particular study only in 2 out of 72 individuals contained non-fibrous plastic, a frequency of 2.8% (Kühn *et al.*, 2018). Seabirds are also known to ingest plastic pollution (Young *et al.*, 2009; Franeker and

Lavender, 2015; Hyrenbach *et al.*, 2017; Rapp *et al.*, 2017). Northern fulmar, *Fulmarus glacialis*, are used as an indicator species for plastic contamination by OSPAR in the North Sea, between 2012 - 2016 of 169 beached fulmar 91 % contained plastic in their stomachs, an average of 22.3 particles with a mass of 0.28 g (Franeker, Rebolledo and Meijboom, 2017). Fulmar are also present in Svalbard, and as sea surface foragers, may be a good bioindicator species for plastic pollution of surface waters, however they are unlikely to reflect the true input of plastic debris to a region as particles in mid- and deep- waters may be different (Choy *et al.*, 2019).

It has been suggested that some of the Arctic microplastic loading could be transported poleward from the North Atlantic via ocean currents. A surface circulation model corroborated with field data suggests that floating debris, including plastic, is carried on the poleward branch of the thermohaline circulation from the North Atlantic to the Arctic (Cózar *et al.*, 2017). The Greenland and Barents Seas in particular have been observed as regions of high plastic abundance which have been observed to have a median value of 6.3×10^4 items km^{-2}, similar to the average number of items in subtropical gyre accumulation zones (Cózar *et al.*, 2017). However, waters in a fjord ecosystem are stratified, particularly in the Summer months, with a surface layer of brackish water due to the large input of glacial and snow meltwater (Svendsen *et al.*, 2002; Cottier *et al.*, 2005). The oceanographic conditions of the fjords of western Spitsbergen are strongly influenced by the currents flowing around the Svalbard Archipelago. The major currents outside the fjord are the local, coastal Arctic-type waters and the West Spitsbergen Current which is the northernmost branch of the Norwegian Atlantic Current (Svendsen *et al.*, 2002; Cottier *et al.*, 2005). The West Spitsbergen Current carries large amounts of salt and heat to the region and may also carry plastic debris; in Kongsfjorden, Atlantic water intrudes far into the fjord in the Summer months however this occupies the sub-surface layers (Cottier *et al.*, 2005). Therefore, it might be predicted that microplastic particles being transported into this area will be more prevalent in these deeper Atlantic influenced waters than at the sea surface.

Here, using a short boat based sampling campaign, I test the hypothesis that there will be higher microplastic concentrations in the deeper Atlantic influenced waters than at the sea surface in the waters of Kongsfjorden, Svalbard.

2.2. Methods

2.2.1. Sampling locations and characteristics

Sampling took place at seven locations in Kongsfjorden, Svalbard during a week in May 2018 (07/05/2018 - 09/05/2018). Work was carried out from the research vessel MV Teisten. These sites were KB0/1, KB2, and KB3, KB5, KB6, and KB7. (GPS coordinates: KB0/1: 79° 00.799 N, 011° 26.000 E; KB2: 78° 58.520 N, 011° 44.390 E; KB3: 78° 57.023 N, 011° 57.446 E; KB5: 78° 54.998 N, 012° 25.710 E; KB6: 78° 55.160 N, 012° 23.259 E; and KB7: 78° 58.112 N, 012° 22.042 E). Locations were based on R/V Oceania Multi-Plankton Sampler stations from (Hop *et al.*, 2002).

At sampling locations, the conductivity, temperature, and depth (CTD) were measured down to a depth of 200 - 300 meters using a CTD probe, SAIV A/S model SD 204. These data were used to inform the deeper water sampling for microplastics (outlined below). It was not possibly to reach sampling station KB7 due to the presence of sea-ice.

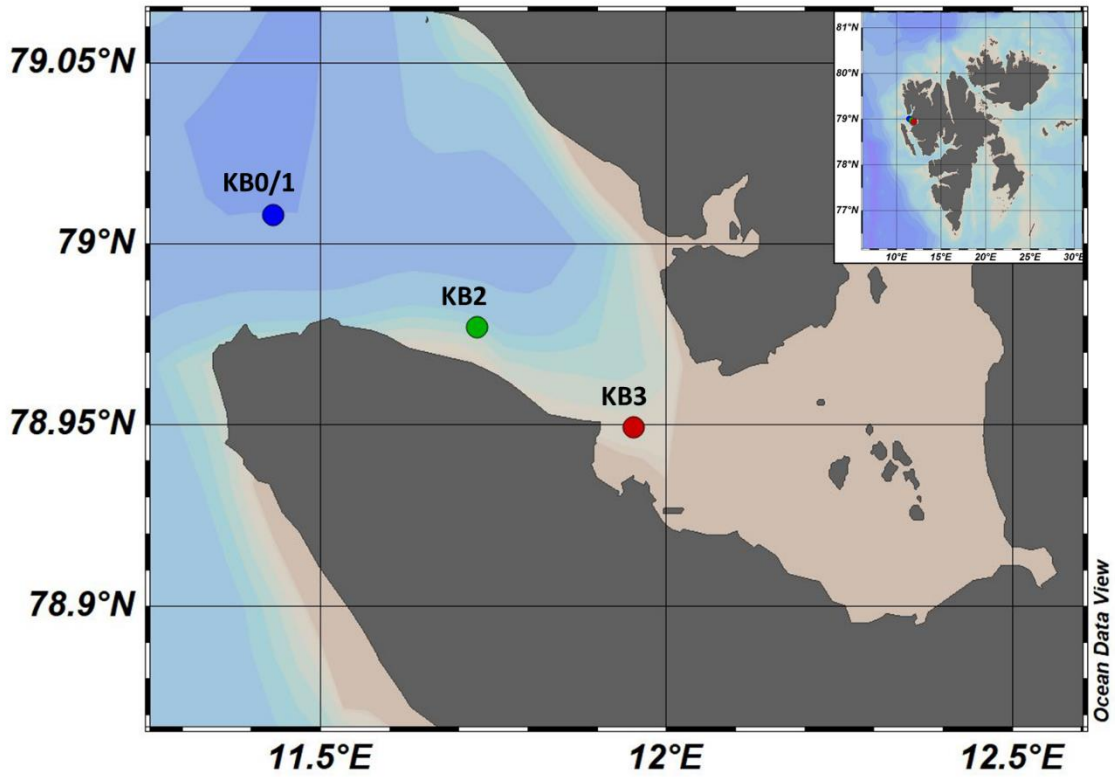

Figure 2.1. Map of Kongsfjorden, Svalbard showing stations KB0/1 (blue), KB2 (green), KB3 (red) the starting location for CTD profiles, surface trawls, and sampling of water at 160 m.

2.2.2. Microplastic sampling

Sea surface microplastics were sampled by surface trawls using a 40 µm plankton net towed from the side of the RV Teisten. Three replicate trawls were performed at each of the three sampling sites. For each sample the net was towed for 2 minutes at an average boat speed of 1.5 - 2.0 knots, this short time limit was necessary due to the rapid saturation of the net with surface plankton. The volume of seawater filtered was calculated by calculating the distance towed using the GPS coordinates taken at the start and end of each tow and extrapolating using the area of the net opening to calculate the cylinder of water filtered.

The content of the net was then carefully rinsed into 500 mL Nalgene sample bottles using a combination of the seawater hose applied to the outside of the net, and tap water filtered to 40 µm for cleaning of the cod end and inner surface of the net.

A deeper water sample was also collected in triplicate for each sampling site. Discrete seawater samples from a depth of 160 m were collected using 10 L niskin bottles. A total of 50 L of sea water was collected using 10 L niskin bottles and emptied onto a 40 µm mesh at the surface, and then carefully backwashed into 500 ml Nalgene bottles as described above using filtered water. At each sampling location one blank sample of filtered water for rinsing the net, fixed with formaldehyde, was taken as a contamination control. Under a laboratory fume hood, all samples (surface and deep) were fixed using formaldehyde to give a final concentration of 4% formaldehyde. Sample bottles were sealed with parafilm and shipped back to the laboratory for analysis.

2.2.3. Sample processing

Deep water samples, which contained far less plankton than surface samples, were filtered directly through 41 µm polyamide nylon mesh (Plastok® Associates Ltd.) with use of a vacuum filter. Surface seawater samples contained a large number of plankton and required further processing to isolate potential microplastic particles. The fixed plankton was allowed to passively settle at the bottom of the Nalgene sample bottles and then the supernatant poured off and filtered through 41 µm nylon mesh. The remaining fraction containing the plankton was allowed dry by evaporation under cover of a fume cabinet, before 50 mL of 70 % nitric acid (HNO_3) was added to each sample bottle. Samples were left for 12 hours at room temperature (~ 20 °C), and then 2 hours at 80 °C, based on similar methods (Lusher *et al.*, 2017). After digestion of the plankton, samples were diluted with pre-filtered water (MilliQ) up to a volume of 1 L (3.5 % HNO_3). Due to the presence of a large quantity of undigested planktonic material which would impair imaging and spectral analysis of sample content, only half (0.5 L) of the diluted sample was filtered for further analysis. Samples were mixed thoroughly, and 0.5 L of the sample was filtered through 41 µm nylon mesh using a

vacuum filter, and then stored in square petri dishes for analysis. The remaining 0.5 L of sample was returned to Nalgene sample bottles for storage.

2.2.4. Imaging and particle analysis

Due to the large number or particles on each filter, the filter area was subsampled. Filters were photographed using a Nikon dissecting microscope at 100 x magnification. Seven photographs of a 0.5 cm x 0.5 cm area were taken from each filter, one from each corner and three from the middle. Suspect particles were then counted and categorised by shape and colour. The mean number of particles per photographed area was used to estimate the number of each particle type on the full area of the filter.

For each category of shape and colour, 10 % of the estimated total particles per filter, with a minimum of 1, were analysed using a PerkinElmer Frontier Fourier-transform infrared (FTIR) spectrometer. Due to the large number of black fragments, a maximum of 3 of these particles were analysed per filter. Spectra were obtained using a PerkinElmer Spotlight 400 FTIR Imaging System (MCT detector, KBr window) operating in reflectance mode and with a wavenumber resolution of 4 cm^{-1}. A total of 16 scans were collected per particle, across a wavenumber range from 4000 to 650 cm^{-1}. All spectra obtained were processed using Perkin-Elmer's Spectrum™ 10 (version 10.5.4.738), enabling normalisation of the data. Polymers were identified by automated matching against commercially available spectral libraries, including Perkin-Elmer's standard Polymers Library and an additional custom spectral library that had been previously prepared in the laboratory through analysis of a range of analytical standards of common plastics. Only match qualities greater than 70 % were accepted for identification purposes. The average match quality of included samples is 80 %. The size of particles was measured using ImageJ software (Schneider *et al.*, 2017).

Contamination during laboratory analysis of samples was controlled for by use of "blank samples". These were "samples" that contained no environmental sample, only filtered water and formaldehyde, that underwent the same method of laboratory analysis as environmental samples including particle isolation techniques and visual

analysis. Two “blanks” were included for each location, one for surface and one for 160 m depth. On average these contained a mean of 11.3 (± SD 6.3) particles, predominantly clear fibres (83 %) but also black fibres (11 %), black fragments (3 %), blue fibres (2 %), and white fragments (1 %). The mean number of particles of each shape/colour that was attributed to post-sampling contamination was subtracted from the final particle counts of each environmental sample.

2.2.5. Data analysis

Great-circle distances of net trawls were calculated using the haversine formula. These were combined with area of the net opening to calculate the volume of water filtered. FTIR spectroscopy results were used to calculate the proportion of particles that could be confirmed as synthetic for individual particle categories (shape/colour) for both surface samples and samples taken at 160 m depth. The proportion of particles that were confirmed as synthetic in the FTIR sub-sample was applied to the numbers of particles on each filter, for an estimated number of synthetic particles for each filter. As FTIR spectroscopy was not possible for the settled planktonic component of the surface samples, the proportions of confirmed synthetic particles from the supernatant of the same samples was applied as an estimate of the proportion of particles expected to be synthetic.

Statistical tests were performed using IBM SPSS Statistics 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY). Maps and T-S plot were made using Ocean Data View, version 5.1.5. (Schlitzer, R., Ocean Data View, odv.awi.de, 2018))

2.3. Results

2.3.1. CTD

For the initial interpretation of the CTD data in the field we looked at measurements of salinity and depth (Figure 2). We thought that salinity could be used to identify Atlantic

water which would be of higher salinity than the surface water. For stations KB1, KB2, and KB3 it appeared that below a depth of ~ 150 m the salinity became less variable, and we used this to inform our deep water sampling depth of 160 m. Data from KB4 and KB5 provided no clear signal for the potential presence of Atlantic water and therefore deeper water layers were not sampled at these locations.

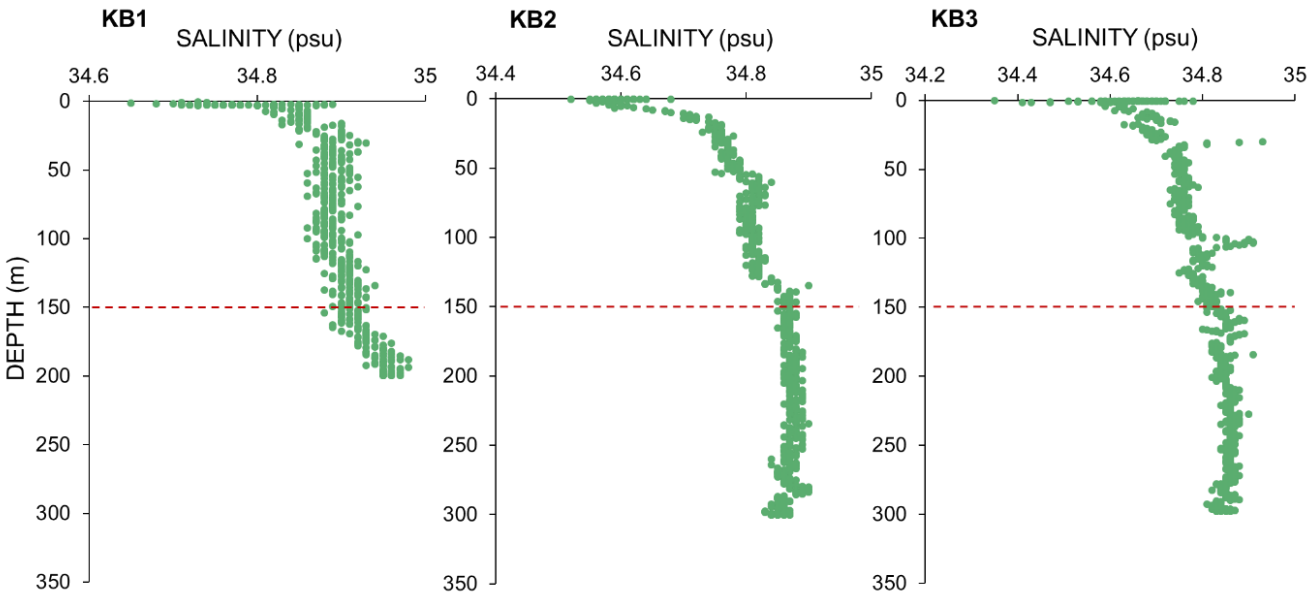

Figure 2.2. Salinity and depth data for sampling locations KB1, KB2, and KB3 in Kongsfjorden, Svalbard. Red dashed line shows 150 m depth. For improved visualisation of the data two "outliers" have been removed from KB1 (data points: 32.14, 0.1; 34.12, 0.31) and KB2 (data points: 32.72, 0.19; 34.35, 0.24).

For later interpretation of the CTD data, water mass definitions are taken from Cottier *et al.*, (2005). Water masses are indicated by the different regions within Figure 2.3. At KB0/1 and KB2, initial surface readings showed a very thin layer of lower salinity water, this is more noticeable at KB0/1 where salinity at 0.1 m was 32.1 psu, however salinity had increased to > 34.3 by ~ 0.4 m depth. As depth increased, temperature decreased to roughly between - 0.5 and 0.5 °C, and salinity and density of the water increased revealing water with properties similar to Arctic Water (AW) or Local Water

(LW) (Fig. 2.3.), from a depth of ~ 0.4 m at KB0/1, and 0.25 m at KB2. At all sites water was relatively homogenous, particularly at KB3 where the surface layer was slightly fresher but not outside the characteristics of Arctic or Local water. Conditions were more consistent at KB0/1, where the water appears to be slightly cooler, denser, and more saline than at KB2 or KB3, slightly closer to the parameters for Winter Cooled Water. For sites KB2 and KB3, salinity and density are relatively consistent below 10 m and 80m, respectively, however the temperature ranges between ~ -0.2 and 0.6 °C.

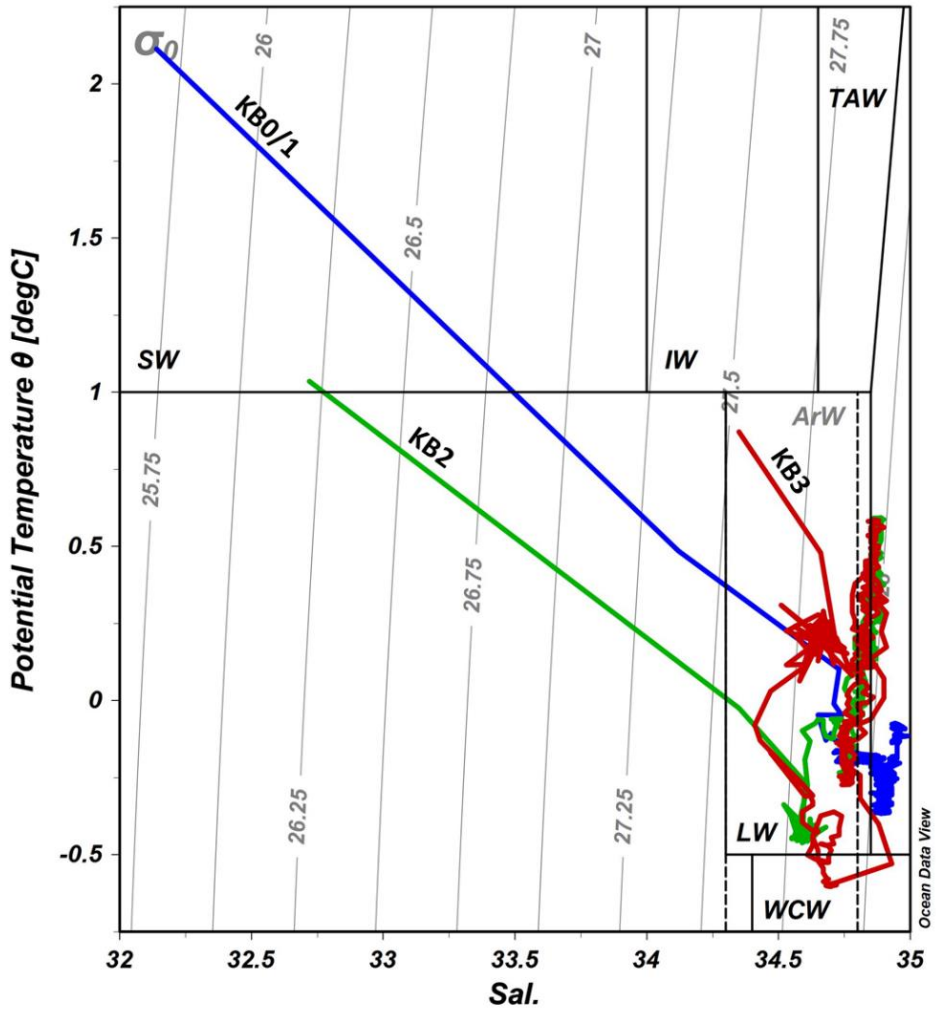

Figure 2.3. Θ-S plot with isopycnols, for the three sampling locations also showing the main water masses in Kongsfjorden, Svalbard. CTD profiles at the sampling locations include stations KB0/1 (blue), KB2 (green), and KB3 (red). Water masses are indicated by areas bounded by solid lines include Surface Water (SW), Intermediate Water (IW),

Transformed Atlantic Water (TAW), Local Water (LW) and Winter Cooled Water (WCW); Arctic Water (ArW) is indicated by dashed lines. Isopycnols are drawn in light grey. Definitions for water masses were taken from Cottier *et al.*, (2005).

2.3.2. Microplastic concentration

The mean number of synthetic particles in water at the sea surface as estimated from sample supernatant ranged from 23.7 ± SD 11.9 particles m^{-3} at site KB3, to 74.1 ± SD 43.9 particles m^{-3} at site KB2. There was no statistically significant difference between total plastic load of surface water between sites KB0/1, KB2 and KB3 ($p > 0.05$). In the settled plankton component of the number of synthetic particles was between 53.8 ± SD 3.2 particles m^{-3} at site KB3, and 92.3 ± SD 12.8 particles m^{-3} at KB0/1. By combining the average number of synthetic particles for sample supernatant and the settled planktonic component total surface water of the fjord was calculated to be between 59.6 ± SD 36.6 particles m^{-3} (KB3), and 149.9 ± SD 50.1 particles m^{-3} (KB2). There was no significant difference in the number of microplastic particles between locations at the surface ($p > 0.05$).

There was a significant difference in the mean number of particles in the surface water compared to the water at 160 m ($t(16) = 4.882$, p-value < 0.000). Surface water samples contained a mean value of only 112.5 ± SD 53.2 particles m^{-3}, compared to deep water which contained a mean value of 2.9 (± SD 1.7) x 10^4 particles m^{-3}. Samples of water at 160 m depth contained a large number of particles, from 1.6 (± SD 0.6) x 10^4 particles m^{-3} at KB0/1, to 4.4 (± 2.3) x 10^4 particles m^{-3} at KB2. There was no significant difference in the number of particles between sites at 160 m depth ($p > 0.05$).

Location	***Microplastic particles m^{-3}***	
	Surface	***160 m***
KB0/1	128 (± 28.6)	1.60 (± 0.62) x 10^4
KB2	150 (± 50.1)	4.43 (± 2.24) x 10^4
KB3	59.6 (± 36.6)	2.52 (± 0.69) x 10^4

Table 2.1. Concentration of microplastic particles m^{-3} in water samples from sea surface and 160 m depth from three sampling locations in Kongsfjorden, Svalbard. Values given to 3 significant figures, ± standard deviation.

2.3.3. FTIR spectroscopy

Spectra generated for plastic particles were relatively high quality matches, a mean confidence of 80.2 % (± SE 0.01). Spectra were generated for 133 particles, 46 from surface samples and 87 from 160 m depth. Of these spectra, 65 were of a library match confidence greater than 70 %. A total of 41 particles were identified as synthetic, see Table 2.2. Polymers and compounds identified were polyethylene, polyester, polyacrylic, polyurethane, polypropylene, polyacrylonitrile, epoxy resin, polyamide resin, polynorbornene, plasticizers, azodicarbonamide, dibutyltin dilaurate. Eleven particles were identified as cellulose, with the exception of one black fibre, all of these were found in surface samples, these were not included in final counts of synthetic particles. We found no statistically significant difference between the types of synthetic polymers present at surface level and 160 m depth (Figure 2.4.) (One-way ANOVA; $p > 0.05$).

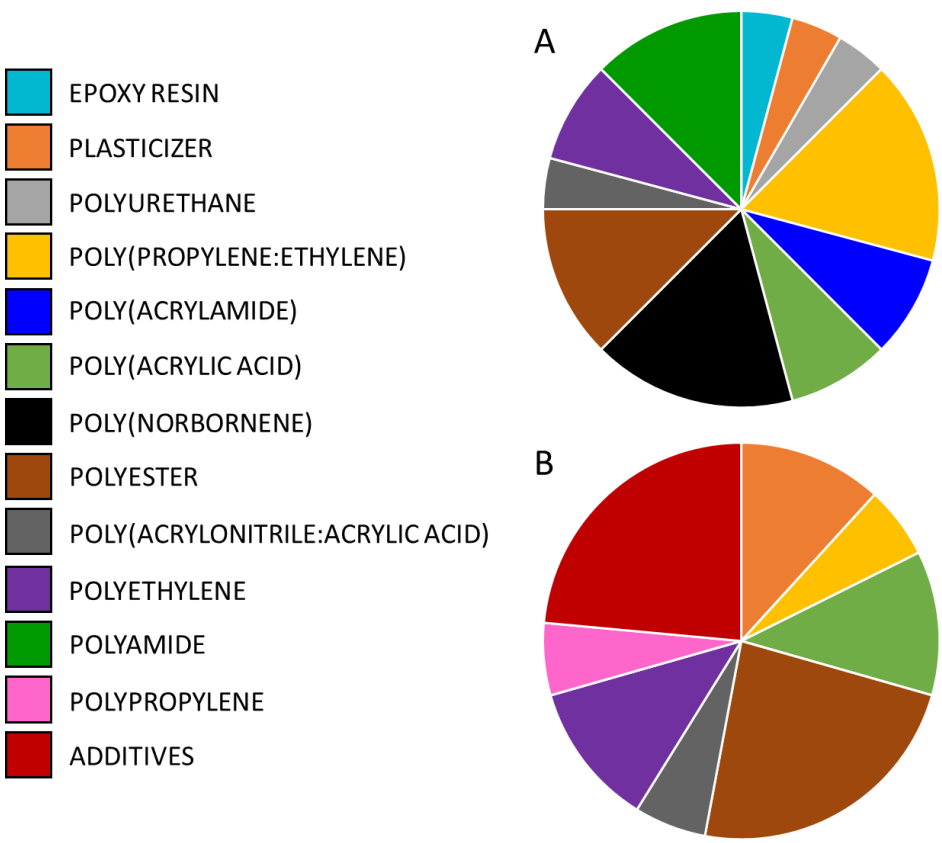

Figure 2.4. Types of synthetic polymers found in water samples from (A) surface sea level, and (B) 160 m depth at sampling stations KB0/1, KB2, and KB3 of Kongsfjorden, Svalbard.

Location	Shape	Colour	Size (um)	Polymer	Confidence
Surface					
KB0/1	Fragment	Black	947 x 463	Epoxy resin	0.87
	Fragment	Pink	116 x 49	Plasthall P-1070 (plasticizer)	0.86
	Fragment	White	210 x 59	Polyurethane	0.86
	Fibre	Blue	849 x 65	Poly(Propylene:Ethylene)	0.84
	Fibre	Black	1763 x 15	Poly(Acrylamide)	0.76
	Fibre	Blue	903 x 37	Poly(Acrylic acid)	0.75
KB2	Fragment	Pink	127 x 73	Poly(Norbornene)	0.91
	Fragment	Blue	851 x 132	Poly(Propylene:Ethylene)	0.91
	Fragment	Clear	383 x 360	Poly(Norbornene)	0.75
	Fragment	Pink	739 x 637	Poly(Norbornene)	0.84
	Fibre	Red	1573 x 95	Poly(Acrylonitrile:Acrylic acid)	0.90
	Fibre	Blue	1705 x 82	Poly(Propylene:Ethylene)	0.86
	Fibre	Clear	1261 x 61	VESTAMID (Polyamide)	0.84
	Fibre	Blue	1954 x 31	Polyester, tere- & isophthalic acids	0.83
	Fibre	Clear	1902 x 34	Polyamide	0.81
	Fibre	Black	927 x 10	Poly(Acrylamide)	0.78
	Fibre	Black	2091 x 15	Polyester, terephthalic acid	0.76
KB3	Fragment	Clear	2325 x 1525	Polyethylene, low density	0.81
	Fragment	Clear	4019 x 1915	Polyethylene, low density	0.87
	Fragment	White	1541 x 161	Poly(Norbornene)	0.73
	Fibre	Black	2143 x 22	Polyester, terephthalic acid	0.90
	Fibre	Blue	1785 x 15	Poly(Propylene:Ethylene)	0.86
	Fibre	Clear	-	Poly(Acrylic acid)	0.81
	Fibre	Black	1210 x 15	Polyamide resin	0.73
160 m					
KB0/1	Fragment	Black	225 x 135	Azodicarbonamide	0.76
	Fragment	Black	110 x 78	Azodicarbonamide	0.74
	Fragment	Black	171 x 167	Azodicarbonamide	0.71
	Fibre	Black	540 x 10	Formic acid	0.77
KB2	Fragment	Red	182 x 92	Alkyd, isophthalic acid	0.80
	Fragment	Blue	142 x 79	Dibutyltin dilaurate	0.72
	Fragment	Blue	144 x 64	Polypropylene	0.87
	Fibre	Red	495 x 13	Polyester, Terephthalic acid	0.78
	Fibre	Black	691 x 35	Poly(Acrylic acid)	0.69
	Fibre	Black	431 x 12	Poly(Acrylonitrile)	0.75
KB3	Fragment	Blue	116 x 25	Poly(Propylene:Ethylene)	0.80
	Fragment	Black	582 x 240	Polyethylene	0.93
	Fragment	Black	120 x 113	Polyethylene	0.83
	Fragment	Red	199 x 176	Plasthall P-1070 (Plasticizer)	0.70
	Fibre	Red	387 x 11	Polyester, terephthalic acid	0.75
	Fibre	Red	1286 x 12	Polyester, terephthalic acid	0.72
	Fibre	Black	1026 x 18	Plasthall 220 (DBEEP)	0.71

Table 2.2. FTIR spectroscopy results of a subsample of particles from water samples taken at 0 and 160 m depth from the sea surface in Kongsfjorden, Svalbard.

2.3.4. Shape of particles

In terms of the percentage representation by particle shape/colour categories, both surface and deeper water samples were dominated by black fragments which constituted 51.7 % (± SD 24.3) and 79.6 % (± SD 7.7) of particles, respectively. Surface waters also contained black fibres (13.9 %), white fragments (13.5 %), blue fibres (10.1 %), clear fragments (3.8 %), red fibres (3.5 %), red fragments (2.8 %), and clear fibres (0.7 %). There were significantly greater proportions of white fragments and blue fibres in surface water compared to deeper water ($t(16) = -7.518$, $p < 0.000$; $t(16) = -4.148$, $p = 0.001$, respectively). No blue fragments were found at the surface. In addition to the black fragmented particles, water from 160 m depth also contained black fibres (15.1 %), red fibres (4.5 %), blue fragments (0.5 %), and red fragments (0.3 %). The water at 160 m depth contained a significantly greater proportion of black, and blue fragments ($t(16) = 3.278$, $p = 0.005$; $t(16) = 4.778$, $p < 0.000$, respectively). There was no significant difference in the mean proportions of red fragments, clear fragments, black fibres, red fibres, and clear fibres between surface and deep water ($p > 0.05$). White fragments, clear fragments, clear fibres, and blue fibres were all absent from samples from 160 m depth.

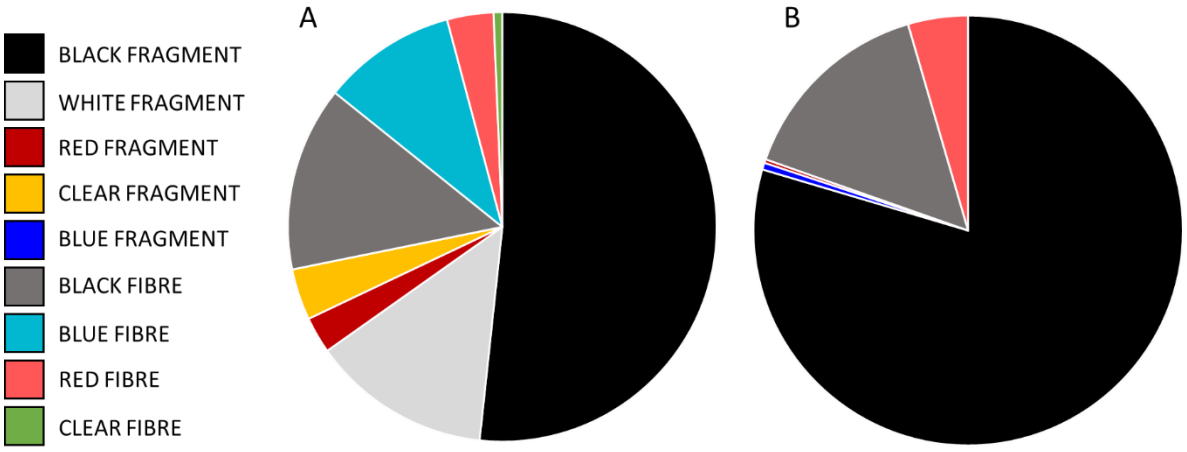

Figure 2.5. Average proportions of microplastic particles categorized by shape and colour in water samples from (A) sea surface level, and (B) 160 m depth at sampling stations KB0/1, KB2, and KB3 of Kongsfjorden, Svalbard.

2.3.5. Size of particles

The sizes of the fragments and fibres, confirmed as synthetic polymers, at the surface and 160 m depth were compared (Fig. 2.6.). Fragments at the sea surface, measured across their largest dimension, were significantly greater in size than those in samples from 160 m depth, an average size of 1126 µm compared to 199 µm ($t(9.232) = 2.365$, $p = 0.42$). The average length of fibres at the sea surface was also significantly greater compared to those at 160 m depth, 1544 µm and 694 µm, respectively ($t(18) = 4.272$, $p < 0.001$).

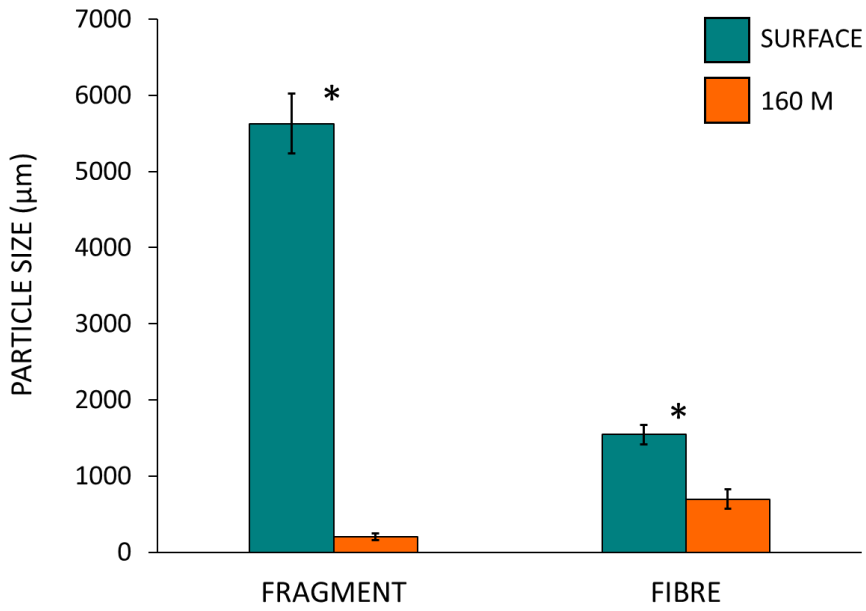

Figure 2.6. Average size of microplastic particles, fragments and fibres, in water samples from surface level and 160 m depth from Kongsfjorden, Svalbard. Asterisks (*) denote the statistically significant difference within each particle shape.

2.4. Discussion

Microplastic pollution was evident in all water samples collected. The sea surface water within Kongsfjorden at an average concentration of 112 (± 53) particles m^{-3}, which is a relatively high number of particles in comparison with estimates and observations from other regions of the Arctic ocean. The concentrations observed in this study are much greater than the 0.34 particles m^{-3} previously reported for surface waters south-west of Svalbard by Lusher *et al.* (2015), and 0.13 particles m^{-3} reported in the Arctic sector of the Pacific ocean (Mu *et al.*, 2019). Cózar *et al*. (2017) reports a maximum value of 3.2 x 10^5 plastic items km^{-2} for the Greenland and Barents seas, and though this is difficult to convert to an accurate concentration by volume it is roughly lower than values for surface water presented here; the maximum surface particle concentration can be roughly converted to 1.1 x 10^7 items km^{-2}. However this region is considered to be a hotspot for microplastic pollution which is increasing over time (Bergmann, Wirzberger, *et al.*, 2017).

Microplastic particle concentrations in seawater collected at 160 m depth were found to be 2 orders of magnitude higher than at the sea surface, with an average concentration of 2.9 (± 1.7) x 10^4 particles m^{-3}. As far as we can tell, sampling of microplastic particles in deeper waters in this region has not been reported before so comparable studies are limited, though these values far exceed values for sub-surface waters (6 m) south of Svalbard, 2.68 particles m^{-3} (Lusher *et al.*, 2015). These concentrations are far more similar to observations from Arctic sea ice cores where highs of 1.2 (± 1.4) × 10^7 particles m^{-3} have been reported Peeken *et al* (2018). These samples were taken from pack ice of the Fram Strait; their lowest reported value of 1.1 (± 0.8) × 10^6 particles m^{-3} was found in a pack ice core from north of Svalbard. However, Obbard *et al.* (2014) reported much lower values in sea ice cores collected across the Arctic Sea, 38 - 234 particles m^{-3} . The sea ice is thought to act as a barrier for the poleward surface transport of microplastic particles, it may be possible that the coastline of Kongsfjorden also halts surface transport and acts as a “trap” preventing further surface transport and allowing plastic to accumulate.

Bergmann *et al* (2017) looked for a correlation between microplastics in deep sea sediment and overlying sea ice, and although they found no significant relationship, they did observe that the two stations with the highest number of microplastics were close to or within the marginal ice zone. They suggest that microplastic from meltwater in addition to those transported on the Thermohaline Circulation may contribute to the high number of microplastics at these locations if the particles sink rapidly and the horizontal displacement is small. Though this is unlikely to contribute to deep sea plastic in Kongsfjorden as typically the fjord is ice-free year round (Svendsen *et al.*, 2002).

In terms of relative abundance these results appear similar to the vertical distribution of microplastics in the pelagic ecosystem of Monterey Bay where microplastic abundance peaked at ~ 200 m depth and contained a greater number of particles than at the surface, albeit with a much smaller difference between means (Choy *et al.*, 2019). The vertical distribution of microplastic particles in waters of the Central Arctic Basin are somewhat the reverse of the findings presented here, where microplastic concentration was much greater in the subsurface Polar Mixed Layer (0 - 375 particles m^{-3}), than at intermediate depths (AW: 0 - 95), and deep or bottom waters (0 - 104) (Kanhai *et al.*, 2018). However, this sampling for this study took place in the much deeper waters of an ocean basin, within the relatively shallow waters of a fjord system local conditions and coastal hydrography will likely play a significant role in determining the concentration of microplastics in waters (Zhang, 2017).

Microplastic particles at the surface were significantly larger than at 160 m depth, particularly fragments which were on average 5430 µm larger, and also fibres which were 850 µm longer than in deeper waters. The accumulation of particles at depth and partitioning of particles by size could be due to interactions between the downward convection of water in the fjord and the relative buoyancy of particles. As WCW is formed as a result of sea ice formation and winter cooling, this dense, cold water sinks to occupy the deeper basins of the fjord (Cottier *et al.*, 2005). It is possible that small microplastic particles of weaker or neutral buoyancy are drawn down to deeper layers by this convection process while larger particles remain afloat. Larger particles have a greater buoyancy due to their larger volume to surface area ratio in comparison to smaller particles, and

the rise velocity of particles has been shown to be dependent on particle size and shape (Kukulka *et al.*, 2012; Fazey and Ryan, 2016; Kooi *et al.*, 2016; Lebreton *et al.*, 2018). This might make large particles more resilient to sinking and therefore more likely to occupy the surface layer.

The majority of particles observed were small black fragments, and due to these being very abundant, only ~ 0.06 % of the black fragments calculated to be on the filters were included in the subsample analysed by FTIR spectroscopy. Of the other particle colours calculated to be present on the filters, ~ 0.6 % of total particles were analysed. A greater number of particles could have been analysed by using an automated method such as those used by Primpke, Dias and Gerdts (2019) or Haave *et al* (2019). Of these black particles only ~ 20 % were identified as synthetic polymer and the ~ 80 % that could not be identified as plastic were not included in the final numbers presented in Table 2.1. Of those that produced FTIR spectra of suitable quality these were identified as polyethylene, epoxy resin, and azodicarbonamide. Azodicarbonamide is a chemical compound which is used as a blowing agent in the manufacture of foamed plastics, although these particles were included in final results based on alternate polymer library matches for all three of these particles as acrylate-acrylamide copolymer (Weber *et al.*, 2016). The difficulty in identification may be due to many of these particles being natural materials as there is a large input of suspended particulate matter naturally occurring in the fjord due to the grinding action of the glacier on the sediment beneath it (Beszczyńska-Moller *et al.*, 1997). These particles look very similar to black particles found by Bergmann *et al.* (2017) in deep sea surface sediments which were mostly small fragments of naturally occurring coal.

The most common polymers identified were polyester (18%), ethylene-propylene copolymer (11.8 %), and polyacrylic acid and polyethylene (10 % each). This is a similar observation to previous sampling of Arctic waters and sea-ice, when excluding cellulose. In waters south of Svalbard polyester and polyamide were the most common plastic polymers (15 % each); and in Arctic sea ice cores polyester (21 %) and polyamide/nylon (16 %) were most common (Obbard *et al.*, 2014; Lusher *et al.*, 2015). Peeken et al. (2018) reports 48 % of particles observed in sea ice were polyethylene. Eleven particles were identified as cellulose, if included in particle counts then these account for 21.2 % of particles.

This is slightly lower than previously reported in Arctic surface waters, 30 %, and arctic sea ice, 54 % (Obbard *et al.*, 2014; Lusher *et al.*, 2015).

Cellulose fibres are not included in the count of synthetic particles as most of the fibres observed were black and this made it difficult to conclude if were dyed which would verify an artificial origin. Modified cellulose fibres in the environment are thought to be mostly sourced from wastewater treatment and the textiles industry, from dyed cotton or rayon (Browne *et al.*, 2011; Friot and Boucher, 2017). However their presence in Arctic waters is interesting as they supposedly have a short lifespan in the marine environment and were found amongst microplastic particles which are theoretically relatively old (Cózar *et al.*, 2017). This might show that they persist long enough in the marine environment to travel great distances from their source. However they may also originate from local sources of contamination or, as these were all found at the surface (with the exception of one black fibre), may have been transported in the atmosphere (Dris *et al.*, 2016). Due to the remote nature of the region and low population density significant local contamination in the region is unlikely, however it is known that a very small amount of microplastic contamination is released in waste water and from other human activities at a similar polar research station, Rothera Research Station in Antarctica (Cózar *et al.*, 2017; Reed *et al.*, 2018).

For FTIR spectral analysis of some synthetic particles (11.8 %), plastic additives were detected rather than the constituent polymer. Some particles were identified as Plasthall P-1070, or Plasthall 220 (DBEEP), these are both branded plasticisers manufactured by Hallstar®. Plasthall 220 (DBEEP) is dibutoxyethoxyethyl phthalate, phthalates are a common additive to plastic polymers particularly polyvinyl chloride (Hermabessiere *et al.*, 2017). One particle was identified as dibutyltin dilaurate, a catalyst used in production of polyurethane, and as a stabiliser in polyvinyl chloride (Davies, 2004). Another particle is reported as formic acid which is a naturally occurring compound, this particle was retained based on a lower spectral library result of “polyacrylic acid” (0.67 confidence) and the physical appearance of the particle. It is likely that the library result of “formic acid” is a reading of surface contamination of the particle by organic materials due to biofouling (Kirstein *et al.*, 2018). Visual identification

by shape and colour are of a lower priority than identification of the constituent polymer, but are also important classification criteria (Hartmann *et al.*, 2019).

CTD profiles were collected for each sampling station to differentiate between different water masses of the fjord. Whilst initially the change in salinity through the CTD profiles was thought to signify a distinct water layer at ~ 150 m depth, on further analysis of the data at a later period I found that the characteristics of the water did not fit within the parameters of Atlantic Water. These are a temperature ≥ 3 °C, salinity ≥ 34.65, and σ_θ > 27.92 (Svendsen *et al.*, 2002; Cottier *et al.*, 2005). As there is no clear signal for this water body in this data set it is not possible to definitively say that the deep water samples are of Atlantic water, and these findings do not provide direct evidence for the hypothesis of long distance transport of microplastic particles in Atlantic water put forward by Cózar *et al.* (2017). However, the microplastic particles observed here are unlikely to originate from local sources due to the low population density, and a surface plastic-to-coastal inhabitant ratio which is much higher than the global average, and suggests long-distance transport as the most likely source (Cózar *et al.*, 2017). The hydrological conditions of Kongsfjorden are largely seasonal, with a major shift in conditions between Summer and Winter months, and Atlantic water may have been detected if the field survey was carried out in the Summer months (Cottier *et al.*, 2005).

In Winter the fjord constitutes two major bodies of water, Local Water and Winter Cooled Water (WCW). During the onset of Spring/Summer conditions these water bodies are modified and shift to characteristics more similar to those of Intermediate Water. At KB3 a relatively cool and dense patch of water was apparent at ~ 25 - 30 m depth which had characteristics similar to WCW, although the water throughout was relatively homogenous, which is typical for the time of year. A previous study of water conditions in the fjord, show that in April water conditions in the fjord are fairly homogenous and weakly stratified, and by June there had been significant modification of water and intrusion of water masses into the fjord mouth (Cottier *et al.*, 2005). The data presented here were collected in May, in comparison with Figure 4 in Cottier *et al* (2005), the CTD profiles appear to fit well at an intermediate point in the seasonal shift of fjord hydrography, where conditions in the fjord have just begun the shift toward their

Summer regime. Summer conditions are characterised by the intrusion of a mix of Atlantic water (AW) and Arctic water (ArW), or Transformed Atlantic water (TAW), and then by full AW from September. Although the switch from an Arctic state (dominated by ArW and WCW) to an Atlantic state (dominated by AW and TAW) can be rapid in June/July. In Kongsfjorden, due to the seasonality of the water body, the transport of microplastic particles into the fjord may also be seasonal, with a greater import of particles in the Summer months with the intrusion of Atlantic water. The particles observed in this study may have been carried into the waters of the fjord in previous years, or in the pockets of modified Atlantic water that begin to enter the fjord as the Winter regime breaks down.

Accumulation of microplastic particles at the surface may be curtailed by local weather patterns which can play a role in surface particle transport (Zhang, 2017). In the days preceding surface sampling, the fjord was subject to heavy south-eastern winds which cleared the fjord of glacial ice. Kongsfjorden has a prevailing south-east wind from the head to the mouth of the fjord, driven by orographic steering of large-scale wind fields and katabatic winds transporting cold dense air from the inland glaciers to the warmer fjord; the prevailing wind direction reverses to an up-fjord direction in the Summer which can impact stratification of fjord waters (Svendsen *et al.*, 2002). The prevailing wind direction and resulting surface currents at the time of sampling may have resulted in surface microplastic debris being pushed back toward the mouth of the fjord and the open sea (Critchell and Lambrechts, 2016). Therefore, the accumulation of particles may be greater at depth because the influence of wind-mediated transport is less apparent.

Evidence is growing of the true abundance of the smallest size fractions of plastic particles in the Arctic, which dominated sea ice and deep sea sediment samples (Bergmann, Wirzberger, *et al.*, 2017; Peeken *et al.*, 2018). The abundance of microplastic particles in this region is important because they are bioavailable to a wide range of species, particularly in the Arctic where copepods dominate. Ingestion of nylon fibres and granules by the North Atlantic copepod *Calanus finmarchicus* has been shown to impact feeding and development, and result in premature moulting (Cole *et al.*, 2019). Exposure to nylon fibres resulted in alterations to prey selectivity and a 40 % decrease in algal ingestion rates.

Additionally, juvenile copepods in the control group experienced significant accumulation of lipids over time, whereas in copepods exposed to nylon granules lipid accumulation was nonsignificant. The impairment of health and developmental processes at the scale of the individual is likely to elicit population level effects and ultimately result in ecosystem scale impacts, particularly for an ecologically important keystone species (Galloway, Cole and Lewis, 2017). However, it is not known whether copepods in the Arctic are consuming microplastic particles, but the cooccurrence of an abundance of both copepods and small microplastics which are rapidly accumulating, means that interactions are likely in the Arctic and should be a focus of future research.

2.5. Conclusion

In this study, I present evidence of the large difference in concentrations of microplastics in water sampled at the sea surface and 160 m depth in Kongsfjorden, Svalbard. I show that the physical characteristics of the collected particles, in terms of size, shape, and colour, are different between the two sampled depths. Microplastic isolation methods and FTIR spectroscopy techniques, for resolution of smaller and a greater number of particles, are highlighted as areas that require development to improve the quality of analysis. Collection of CTD data and microplastic samples later in the year when Atlantic water fully occupies the fjord would be required to differentiate between the plastic content of Arctic and Atlantic waters with certainty and suggest whether microplastics are transported on Atlantic waters to this region of the Arctic.

Chapter 3

Particle characteristics of microplastics contaminating the mussel *Mytilus edulis* and their surrounding environments.

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Whilst this chapter is my own work, I received the following inputs from my co-authors:

My supervisor Dr Ceri Lewis contributed to experimental design, field work, data analysis and editing of the manuscript for publication.

Adam Porter: field work assistance, guidance in techniques for sample processing, microplastic isolation, and analysis in the laboratory.

David Santillo: guidance with use of the PerkinElmer FTIR spectrometer and spectral analysis techniques.

Holly Simpson and Sophie Lloyd-Williams: assistance with sampling and data collection in the field and laboratory.

Abstract

We investigated the relationships between the environmental partitioning of macro-, meso- and microplastics and their uptake into the mussel, *Mytilus edulis*. Sediment samples, overlying seawater and mussels from 9 intertidal locations in the South West of England were analysed for abundance and type of microplastic. Micro- and mesoplastic-like particles were found in 88.5% of the 269 mussels sampled, ranging from 1.43 to 7.64 items per mussel. Of these plastic particles, 70.9% were identified as semi-synthetic (mainly modified-cellulose). Mussel microplastic abundance, but not polymer type, was correlated with that of their surrounding sediment, but not with sea-surface microplastic concentration or mussel size. We found significant differences in the relative abundance of polymers and particle sizes between seawater, sediment, and mussels, with mussels over-representing modified cellulose fibre abundance but under-representing polyvinyl. Hence, the particle characteristics of mussel microplastic contamination are not directly proportional to that of the microplastics in their surrounding environment.

3.1. Introduction

There has recently been a dramatic rise in public awareness, policy and scientific focus on plastic waste, particularly in single-use consumer products and the role of microplastic as an environmental pollutant. Between 4.8 to 12.7 million metric tonnes of plastic are thought to enter the marine environment each year (Jambeck *et al.*, 2015), resulting in an estimated 93 - 236 thousand metric tonnes of microplastic particles floating on the sea surface (Van Sebille *et al.*, 2015). Plastic pollution is a global issue, with macro and microplastics now known to be present throughout both freshwater and marine ecosystems from the Arctic, to the tropics and coral reefs, and the deep sea (Hall *et al.*, 2015; Courtene-Jones, Quinn, Gary, *et al.*, 2017; Cózar *et al.*, 2017). The definition of microplastic debris was originally arbitrarily proposed as any plastic particle < 5 mm, (Arthur *et al.*, 2008) but it has recently been suggested this should be re-defined as particles 1 to < 1000 µm, with particles 1 mm to 10 mm now being referred to as mesoplastics (Hartmann *et al.*, 2019). Plastic debris comprises a complex mixture

of particles which are often categorised by visual characteristics such as size, colour, and shape, and is a relatively diverse pollutant, covering a wide range of sizes and shapes from larger beach litter items down to the nano- scale, and a range of different buoyant and non-buoyant polymer types (Hartmann *et al*., 2019). Micro- and mesoplastic particles fall within the size range of the optimal prey species for many animals at the base of the marine food web (Galloway *et al*., 2017) with increasing evidence of their ingestion by a wide range of species from zooplankton (Desforges, Galbraith, and Ross, 2015) to marine mammals (Nelms *et al.*, 2019). This combined with their prevalence and persistence throughout marine ecosystems has raised concerns globally over their potential impacts to marine species.

Globally, coastlines are diverse habitats supporting an abundance of ecologically and economically important marine species. Coastal microplastic pollution has been shown to vary by region and is dependent on a wide variety of factors such as oceanic currents, local tides and geography (Jambeck *et al.*, 2015), but typically microplastic concentrations are high, likely due to the constant land-based input. Although plastic pollution is ubiquitous in the marine environment and can travel long distances from its sources, localised sources such as wastewater effluent and poor waste management from coastal urban populations contribute a significant component of coastal microplastic pollution (Jambeck *et al.*, 2015; Graca *et al.*, 2017). Hence the risk of biological uptake of microplastics in coastal regions is thought to be relatively high (Clark *et al.*, 2016; Graca *et al.*, 2017). Whilst data on the sea-surface distribution and abundance of microplastics has increased greatly in recent years, our understanding of the movement of plastic particles away from the surface, through marine ecosystems and their ultimate fate in the marine environment remains limited. Processes such as biofouling, ingestion and subsequent incorporation into faeces, and eventual aggregation with organic matter (Zhao *et al.*, 2018), all influence the buoyancy of plastic particles (Galloway *et al*., 2017) leading to the recent paradigm that most plastic eventually sinks to the benthos (Koelmans *et al.*, 2017). Hence, benthic sediments may be a major sink for plastic particles (Woodall *et al.*, 2014; Kaiser, Kowalski and Waniek, 2017; Porter *et al.*, 2018). Along the coastline, where many benthic species feed, particles may also be re-suspended by turbulent currents and bioturbation, potentially keeping these microplastics bioavailable to benthic

feeders. Microplastics are known to be readily ingested by a range of marine species including pelagic and benthic fish and invertebrates, hence benthic coastal species may be at greater risk from plastic contamination (Lusher, McHugh and Thompson, 2013; Rummel *et al.*, 2016; Graca *et al.*, 2017; Halstead *et al.*, 2018). Understanding the local factors that influence biological uptake of microplastic by coastal benthic species is critical to being able to assess the risk that this pervasive pollutant poses to these important ecosystems (Seitz *et al.*, 2014).

The mussel, Mytilus edulis, is a keystone coastal species with important roles in ecosystem functioning; including habitat formation for diverse benthic communities (*Joint Nature Conservation Committee*, 2008) and nutrient recycling. They play an important role in benthic-pelagic coupling by removing large quantities of suspended organic matter from the water by filter-feeding, and through the production of faeces and pseudofaeces (Ward and Shumway, 2004) and process large volumes of water; for example under optimal algal conditions a 21.5 mm sized mussel will filter an average of 15 mL min-1 (Riisgård, Egede and Barreiro Saavedra, 2011). Coupled with their wide geographical range and low metabolic transformation rates, these traits make mussels useful in monitoring programmes as effective small stationary water samplers for many potential pollutants and dissolved chemical contaminants. The relationship between the level of waterborne contaminants and bivalve tissue concentrations is well established, for example in the NOAA Mussel Watch Programme which monitors over 150 organic and inorganic contaminants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), the pesticide dichlorodiphenyltrichlorethane (DDT) (*National Oceanic and Atmospheric Administration*, 2018). It has been suggested by a number of studies that mussels can also be useful biomonitoring tools for evaluating environmental microplastic pollution (Beyer *et al.*, 2017; Bråte *et al.*, 2018; Li *et al.*, 2019), however the properties of particles within the definition of “microplastic” are complex and hence their uptake into biota from the environment may not follow the same relationships or behaviours as dissolved chemicals and or their derivatives.

Microplastic uptake by mussels is well established, both in laboratory studies (Browne *et al.*, 2008; Van Cauwenberghe *et al.*, 2015) and in their natural habitats

and may occur by ingestion or adherence to tissues (Kolandhasamy *et al.*, 2018; Qu *et al.*, 2018), with numerous studies now reporting microplastic contamination of wild mussels (De Witte *et al.*, 2014; Li *et al.*, 2016; Santana *et al.*, 2016; J. Li *et al.*, 2018; Phuong *et al.*, 2018; Qu *et al.*, 2018; Zhao *et al.*, 2018). Trophic transfer of microplastics ingested by mussels has also been demonstrated under laboratory exposure scenarios, providing a route through which microplastic particles can be accumulated and enter the food chain (Farrell and Nelson, 2013). Mussels are also economically important food species, accounting for more than a third (roughly 470 thousand tonnes) of production by weight of the aquaculture industry in the European Union (*Eurostat.*, 2016). Hence microplastic ingestion by mussels is of additional concern for its human health implications in a species which we consume whole without removing the guts (Van Cauwenberghe and Janssen, 2014).

Here, we investigate the relationship between the abundance and type of microplastic particles in mussels, and that of the mussels' immediate environment, via surveys of 10 mussel populations at 9 locations across the South West coast of the United Kingdom. We include an assessment of the larger beach macroplastic debris at each location to assess whether there is any similarity in composition between the larger litter items and smaller microplastic items of beach plastic debris for each site. Understanding the relationship between environmental plastic contamination and microplastic uptake in mussels is key to assessing the risk that microplastic pollution poses to their ecological functions and their human consumers to be accurately assessed, as well as assessing their application as biomonitoring tools for microplastic pollution.

3.2. Materials and Methods

3.2.1. Site selection

Sampling took place at 9 locations on the South West coast of U.K. during the August - December period in 2017 (mussels only) and 2018 (seawater, sediment and mussels, see SI Fig. S1 for a map of the locations and their latitude and

longitude). Crooklets beach, Barricane beach, Constantine Bay, and Port Gaverne were sampled in 2017; Starcross, Yelland Quay and Trebarwith Strand were sampled in 2018. Torquay and Whitsand Bay were sampled in both 2017 and 2018. The sampling sites are mostly rocky shore beaches, with the exceptions of Starcross and Yelland quay which are estuarine habitat on the river Exe and Taw estuaries, respectively.

3.2.2. Water sampling

Surface seawater was sampled in triplicate for each site using a 53 µm plankton net, towed through surface water for three minutes within 10 m of the waterline, at a minimum depth of 25 cm to allow full submersion of the net. GPS coordinates were recorded at the start and the end points of each trawl (Garmin GPSMAP® 78s) to calculate the distance of the trawl. The contents of the net were then thoroughly rinsed into 0.5 L Nalgene sample bottles using MilliQ, ultra-pure water filtered to 0.22 µm. Samples contained suspended sediment and organic matter which was allowed to settle in the bottles, then the supernatant was then filtered through 50 µm polyamide nylon mesh (Plastok® Associates Ltd.) using a vacuum filter in a laminar flow hood to reduce atmospheric contamination. Microplastic-like particles were removed from this sediment by $ZnCl_2$ density floatation separation, using the method for sediment analysis detailed below, then filtered through the same mesh as the respective supernatant. Filters were stored in sealed square petri dishes until analysed (below).

3.2.3. Sediment collection and density separation

Three sediment samples were collected at each site, one from within the strand line, one from the middle of the beach, and one close to the low tide mark. Sediment was collected adjacent to the mussel beds by taking the surface 1 cm of sediment from within a 1 m^2 square quadrat with a metal trowel. Sediment samples were then stored in clean plastic sample bags at -20 ºC until analysed. Defrosted sediment was placed into 1 L beakers and then into a drying oven at 60 °C overnight. From each of these samples (three per site), a further three 50 g sub-samples of dry sediment were then taken for the isolation of microplastics,

resulting in a total of 450 g of analysed sediment per sampling site. Whilst this is a relatively small amount of sediment to analyse per site, this allows the use of Sediment-Microplastic Isolation (SMI) units, custom-built according to the design and methods developed by Coppock et al. (2017), to separate potential microplastics from the sediment with a high recovery efficiency (95.8 %). This technique allows better recovery of the smaller particles. A pre-filtered (50 µm) $ZnCl_2$ solution at a density of 1.5 g cm^{-3}, was chosen as a floatation media based on its effective recovery of dense polymers. The $ZnCl_2$ sediment solution was filtered through 50 µm polyamide nylon mesh using a vacuum filter and stored in sealed square petri dishes until analysed (below).

3.2.4. Mussel sampling

Thirty mussels were collected from each site (269 sampled in total, mean length 41.6 mm ± SD 12.7, 29 mussels from Starcross) selected to cover a wide range of mussel sizes, positions and orientations of the mussels on the substrate and within the site. Mussels were stored in plastic sample bags and stored in a freezer at -20 °C until dissection. All subsequent work was carried out inside a laminar flow hood to minimise airborne contamination. Once defrosted, the width and length of the shell of each mussel was measured and then thoroughly rinsed with MilliQ to remove external microplastic contamination. Mussel soft tissue was then excised, and wet weight measured. During this process samples were covered with foil to avoid airborne contamination. Mussel tissue was then digested at 70 °C oven in 10% potassium hydroxide until fully digested, up to 48 hours (within the range of conditions used in previous studies, reviewed by Lusher et al., (2017). The contents of each sample were filtered through 50 µm nylon mesh using a vacuum filter. Filters were stored in sealed petri dishes until further analysis.

3.2.5. Beach litter survey

Large plastic items were collected within a 100 m section of the beach, from the low tide mark to the back of the beach. All visible plastic was collected within an

upper time limit of 90 minutes and standardised to the number of participants involved. Collected items were categorised using the OSPAR guideline for monitoring marine litter on beaches (*Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area*, 2010). We removed 10% of items of each category, minimum of 1 item, for FT-IR spectrometry analysis.

3.2.6. Analysis of filters and FT-IR analysis

Filtered material was analysed visually using a dissecting microscope at 30x magnification. Potential microplastic particles were counted and classified by shape and colour, and 10% of each category, with a minimum of three particles, were removed and stored for spectral analysis. To account for any contamination of laboratory origin, procedural blanks were performed (6 per site for mussel, and 1 per site for water and sediment samples) that underwent the same processing as water, sediment, and mussel samples but did not contain a sample. On analysis, blank samples included only fibrous particles, which is likely airborne contamination from clothing. Mussel sample blanks contained on average 1.86 ± 0.28 black fibres, 1.62 ± 0.33 clear fibres, and 0.12 ± 0.05 red fibres. The mean number of particles for each particle category (shape and colour) across the blanks were subtracted from all data prior to data further analysis and is not included in any data presented.

Potential microplastic particles were analysed using a PerkinElmer Frontier Fourier-transform infrared (FT-IR) spectrometer. For larger pieces that could be easily handled, FT-IR analysis was carried out using a universal diamond –ATR attachment. For the majority of smaller pieces FT-IR spectra were obtained using a PerkinElmer Spotlight 400 µFT-IR Imaging System (MCT detector, KBr window) operating in reflectance mode and with a wavenumber resolution of 4 cm^{-1}. A total of 16 scans were collected, across a wavenumber range from 4000 to 650 cm^{-1}. Spectra were then processed using Perkin-Elmer's Spectrum™ 10 (version 10.5.4.738), enabling normalisation of the data and base-line correction. Polymers were identified by automated matching against commercially available spectral libraries, including Perkin-Elmer's standard Polymers Library. Only match qualities greater than 70% were accepted, with an average match quality

of samples of 85%. Particles were photographed using the spectrometers imaging software and the lengths of fibres and fragments then measured using ImageJ 1.47v (Schneider *et al.*, 2017). Prior to data analysis, particle categories which could not be confirmed as synthetic by μ-FTIR spectrometry, were excluded. This included “film” in which all particles examined were confirmed as chitin, and “white beads” which were all confirmed as calcium carbonate mussel pearls. Larger plastic pieces from the beach litter survey were analysed using a Cary 630 FTIR spectrometer (Agilent Technologies). Samples were prepared for analysis by removing the degraded and biofouled surface layer with a razor blade to improve the quality of the spectra. Biofilms have been shown to mask the distinct identifying peaks of synthetic polymers (Ghosal *et al.*, 2018).

3.2.7. Data analysis

Data presented is based on the confirmed anthropogenic particles following FTIR analysis. Statistical analyses (ANOVA and linear regression) were performed on data corrected for contamination found in procedural blanks using SPSS Statistics 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY). Differences between total number of particles in seawater, sediment, and mussel samples were determined using One-Way ANOVA with a Tukey’s post hoc test. Linear regressions were used to determine the relationship between microplastic in mussel tissue, seawater and sediment. Linear regression was also used to determine the relationship between mussel size and microplastic particle abundance. Statistical significance was accepted at p-value < 0.05.

3.4. Results and Discussion

Microplastic contamination of seawater, coastal sediments, and mussels was evident at all of our sampling locations across the South West of the U.K. All surface seawater samples contained microplastic particles, with concentrations ranging from 1.97 to 3.38 particles m-3, but with no significant differences in

seawater concentrations of these particles across our study sites (Fig. 1a, one-way ANOVA, $F_{4,10} = 0.228$, p-value = 0.916). Of these floating particles, 51 % were microfibres and 47 % were fragments, with only 0.03 % comprising microbeads. Microplastic contamination of the surface layer of intertidal sediment did differ significantly between locations (Fig. 1b, one-way ANOVA, $F_{4, 10} = 4.544$, p-value = 0.024), with concentrations ranging from 33.9 particles kg-1 at Torquay to 402.0 particles kg-1 at Whitsand Bay. The majority of these particles were microfibres (93 %), with only 7 % being fragments, found in samples from only three of the five sites analysed for sediment. No microbeads were observed in the sediment samples analysed from our study sites.

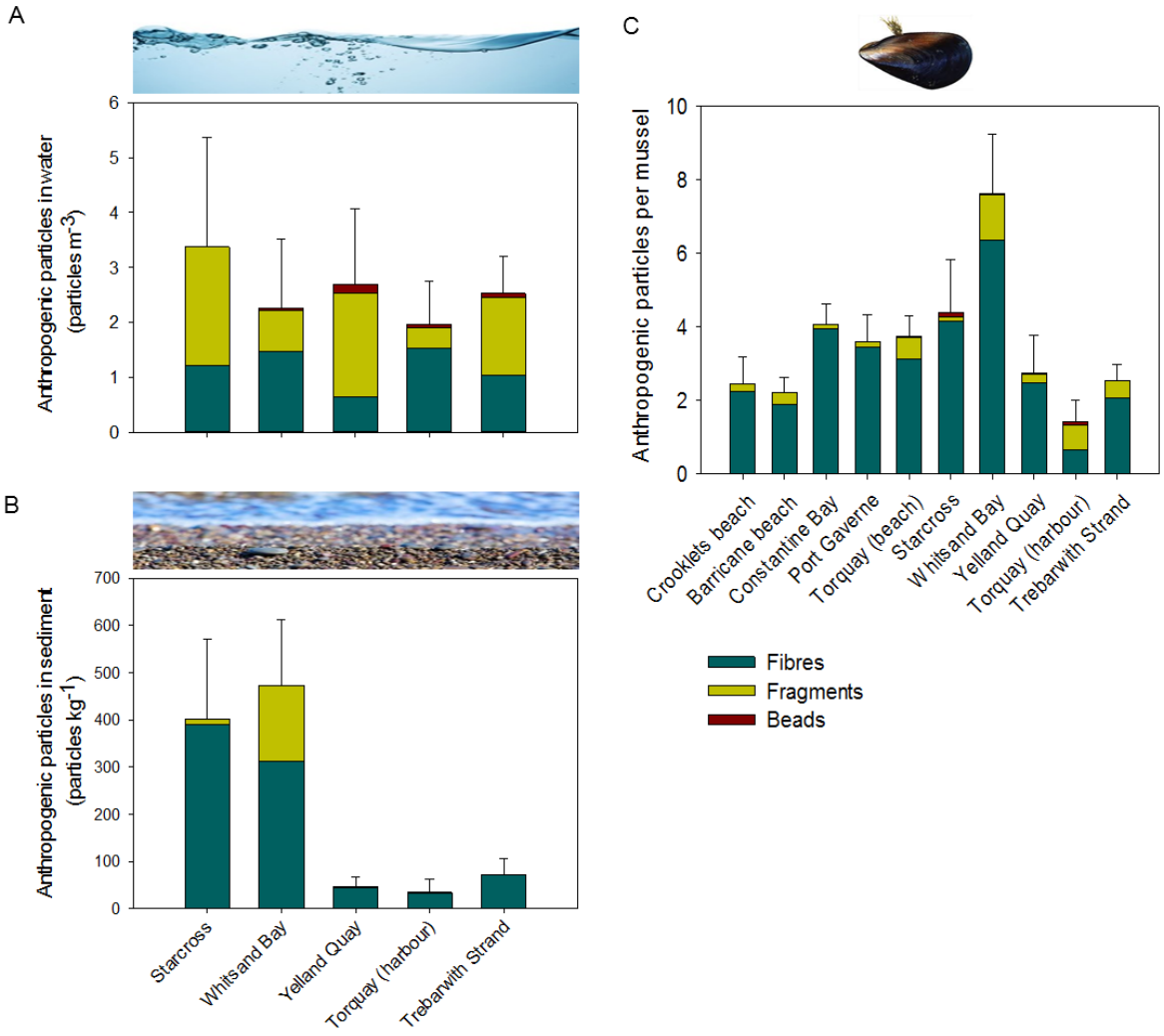

Figure 3.1. The average number of microplastic-like particles, characterised according to shape found in (A) surface seawater (2018 data) (B) the surface 1 cm of sediment (2018 data) and (C) within the tissues of the mussel *Mytilus edulis* (2017 and 2018 data) at coastal sites in Devon and Cornwall, SW England. Data as mean ± standard error.

Microplastic particles were found within 238 of the total 269 mussels sampled (i.e. 88.5% of mussels) across the 10 mussel populations studied (from 9 locations; two different populations were sampled within Torquay Bay) (Fig. 1c). This particle load per mussel differed significantly between sites (One-way

ANOVA; F9, 259 = 4.018, p-value < 0.001 Fig. 1c), with mussels from Whitsand Bay containing the highest average particle loads of 7.64 ± 1.61 particles per individual, and Torquay (harbour) the least, with 1.43 ± 0.30 particles per individual. Of these particles, 87 % were microfibres whilst 12 % were fragments. Only 9 microbeads were found within mussels across all sites sampled (< 1 %). These numbers of microplastic particles per individual mussel are similar to the range reported in a previous study on microplastic contamination of mussels in the U.K. (1.1 - 6.4 items per individual) and are similar to those reported in China (Li *et al.*, 2018) and Norway (Bråte *et al.*, 2018). However, they are higher than the contamination levels reported for mussels in other studies from Belgium, Germany, French and Dutch coastal waters (De Witte *et al*., 2014; Van Cauwenberghe *et al*., 2015; Van Cauwenberghe and Janssen, 2014). The highest numbers of microplastics reported for mussels to date is that reported for mussels collected from a beach in Nova Scotia, Canada, where 34 - 178 items/individual was recorded, mostly comprising microfibres (Mathalon and Hill, 2014).

Micro-FTIR spectroscopy was conducted on 247 randomly selected particles from across the seawater, sediment and mussel samples. This analysis revealed that 33.9 % of these particles were synthetic plastic polymers, mainly polystyrene, polyethylene and polypropylene (Fig. 2). Particles of natural origin, 9.3 % of items analysed, and spectra with a low match quality (below 70 %) were discarded from our final results and are not presented in our data. A large number of particles (56.8 %), were semi-synthetic fibres comprised of modified-cellulose. Potential rubber fragments were also found in some samples but are not included in the data presented due to difficulties in generating high quality FTIR spectra from these particles. The modified-cellulose fibres were mostly black/blue or red and hence are likely to be viscose/rayon fibres from textiles, therefore we include these within our counts as these highly modified natural polymers have been included within the recent ‘microplastic’ definition suggested by Hartmann et al., (2019) due to their artificial composition.

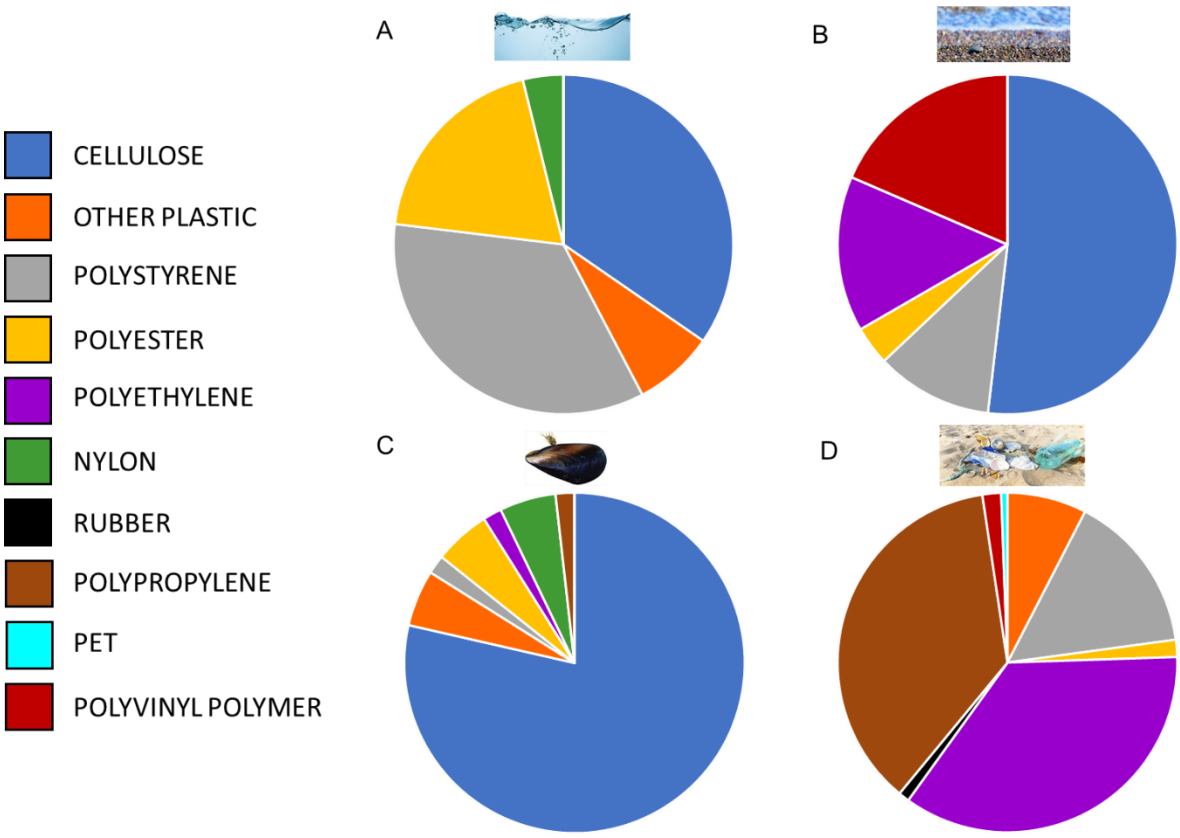

Figure 3.2. Results of ATR/FT-IR spectral analysis, showing proportions of polymers of anthropogenic particles present in (A) samples of seawater, (B) the surface 1 cm of sediment, (C) within *Mytilus edulis*, and (D) macroplastic beach debris from coastal sampling sites in Devon and Cornwall, SW England.

This follows an emerging trend for studies in coastal areas where particles are subsequently analysed using µFT-IR or other spectral techniques such as Raman, which often find a high percentage of anthropogenic particles in seawater or ingested by marine species comprise modified-cellulose-based anthropogenic materials such as viscose or rayon (Remy *et al.*, 2015), or natural fibres such wool or cotton (Courtene-Jones, Quinn, Murphy, *et al.*, 2017; Halstead *et al.*, 2018; J. Li *et al.*, 2018). For example, Bråte *et al* (2018) reports cellulose fibres as the dominant particle in mussels on the Norwegian coast, whilst a recent global study found that 57% of the microfibres isolated from marine samples are classified as synthetic, 12% as semi-synthetic, and 31% as non-synthetic (Barrows, Cathey and Petersen, 2018). According to the recent Hartmann *et al*. (2019) review, synthetic-cellulose fibres should be considered within the definition of 'plastic debris' due to their highly modified and persistent nature, however distinguishing between synthetic and natural cellulose-based fibres using currently available µFT-IR spectral libraries can be challenging, making categorising these fibres as either plastic or non-plastic particles problematic.

A variety of synthetic and semi-synthetic polymers were found across the different environmental compartments that we studied (i.e. seawater, sediment, mussels), however these were not all distributed equally across compartments, i.e. environmental partitioning of polymer types was observed which may influence what is bioavailable to a benthic mussel to ingest. For example, modified-cellulose made up significantly more of the particles found in mussels than in the overlying seawater or the beach litter (One-way ANOVA; $F_{3, 22} = 19.282$, p-value < 0.000, Fig. 2). The buoyant polymer polyester (7.5 % of total) made up a significantly greater proportion of particles in the overlying seawater than those in the sediment or in the beach macroplastic items (One-way ANOVA; $F_{3, 22} = 5.990$, p-value = 0.004, Fig. 2 b). There was a significantly greater proportion of polyvinyl polymers in the sediment than in mussels or the overlying seawater (One-way ANOVA; $F_{3, 22} = 8.039$, $P = 0.002$). Other polymers identified include polystyrene (11.0 %), polyethylene (3.4 %), polyvinyl-based polymers (4.2 %), nylon (2.5 %), modacrylic (1.7 %), and polypropylene, polyacrylamide, ethylene/acrylic acid, and plasticizer (0.85 % each) (Fig. 2a, b, c).

Macroplastic pollution of the strandline and intertidal zone was evident at all of our sampling locations but varied greatly in abundance from site to site. We collected a total of 7,411 beach macroplastic debris items, of which 3,723 items were collected from Whitsand Bay, accounting for more macroplastic items than the sum of all other locations. Trebarwith Strand was the least littered site with only 17 items collected. Macroplastic beach litter was diverse in composition but was dominated by fragmented plastic debris with pieces 0 – 2.5 cm and pieces 2.5 – 50 cm making up 44.8 % and 35.0 % of total collected items by number, respectively, consistent with previous beach litter studies for the U.K. (Nelms *et al.*, 2017; Watts *et al.*, 2017). Other items (< 5 % each) were mostly consumer products such as food and cosmetic item packaging and containers, ropes, cigarette lighters, and plastic bags. 811 of these macroplastic items were analysed using FTIR, with an average certainty of 85.4 % in order to compare these polymer types with the composition of the microplastic particles found at the same locations and within the mussels. Despite a large variety in litter items, the macroplastic was dominated by only two buoyant polymers, polyethylene (35.4 %) and polypropylene (36.6 %), representing a significantly greater proportion than found at the micro- scale (One-way ANOVA; $F_{3, 22} = 7.747$, p-value = 0.001; $F_{3, 22} = 20.814$, p-value < 0.001, respectively, Fig. 2 d). The remaining items comprised polystyrene (15.3 %), polyvinyl polymers (1.78 %), polyester (1.63 %), rubber (1.0 %), polyethylene terephthalate (0.6 %) and 'other' plastic polymers (7.6 %), (Fig. 2 d).

These large differences in the polymer composition of large macroplastic litter on beaches and the microplastics found in the same sediments, the nearby surface seawater and within the mussels suggests that there is no direct relationship between the two size fractions of debris, i.e. the larger macroplastics litter items are not the source of the smaller items on the same beach. The local coastal topography, sediment type, and hydrodynamics, in addition to particle characteristics are all likely to play a role to produce the mix of plastic items that accumulate on any section of coastline (Zhang, 2017). The fragmentation of coastal macroplastic debris might produce particles with altered physical characteristics from the original larger items which are then influenced differently by local physical factors. Particle shape, size, and density may determine a particles position in the water column and changes to these characteristics could

determine the way in which the particles are transported (Kowalski, Reichardt and Waniek, 2016; Lebreton *et al.*, 2018). Whether a particle is in suspension or settled in the sediment could determine to what degree it affected by surface currents and turbulence, wind and wave induced drift, or benthic sediment transport dynamics such as bed-load or suspended-load (Ballent *et al.*, 2013; Zhang, 2017). Ultimately, changes to a particle's physical characteristics could result in transport away from the site of origin. This may explain why we find such a high percentage of polypropylene and polyethylene at the macro- scale, but not at micro- or meso-scales at these intertidal sites.

No relationship was found between the total number of anthropogenic particles in individual mussels and mussel wet weight (g) or any other parameter of individual size tested (Fig. 3.). This is in contrast with previous findings of Bråte *et al* (2018) who did find a relationship between mussel size and number of particles ingested in their study of Norwegian mussel populations. Studies in microplastic uptake often attempt to normalise their measures of plastic particles per individual by mass, following an assumption that size influences uptake rates in a similar way to respiration rates (Hamburger *et al.*, 1983) and feeding rates (Riisgård, Larsen and Pleissner, 2014), however the results presented here suggests that this relationship does not exist for microplastic uptake in the case of M. edulis at this particle size range of microplastic contamination. Whilst mussel condition varies seasonally and hence shell length may be considered a more reliable indicator of filtration rate than tissue weight (Riisgård et al., 2014), similarly there was no relationship between shell length and microplastic uptake. Little dose response data exists for microplastic uptake for any marine species, particularly at these lower environmental concentrations. Microplastics can also pass through guts and be egested with the faecal material (Cole *et al.*, 2016), so may only ever be present within an individual for a short time related to the gut passage time of that individual. This may be shape dependant with fibres potentially being more likely to be retained but the evidence supporting this idea is currently limited. Hence the dynamics of particle uptake and body load may not scale with size at these low concentrations but rather be driven by particle encounter rates influenced by localised seawater movement at microscales. Thought needs to be given to the rationale behind transforming microplastic body load by individual body size (mass) where this is done in the future.

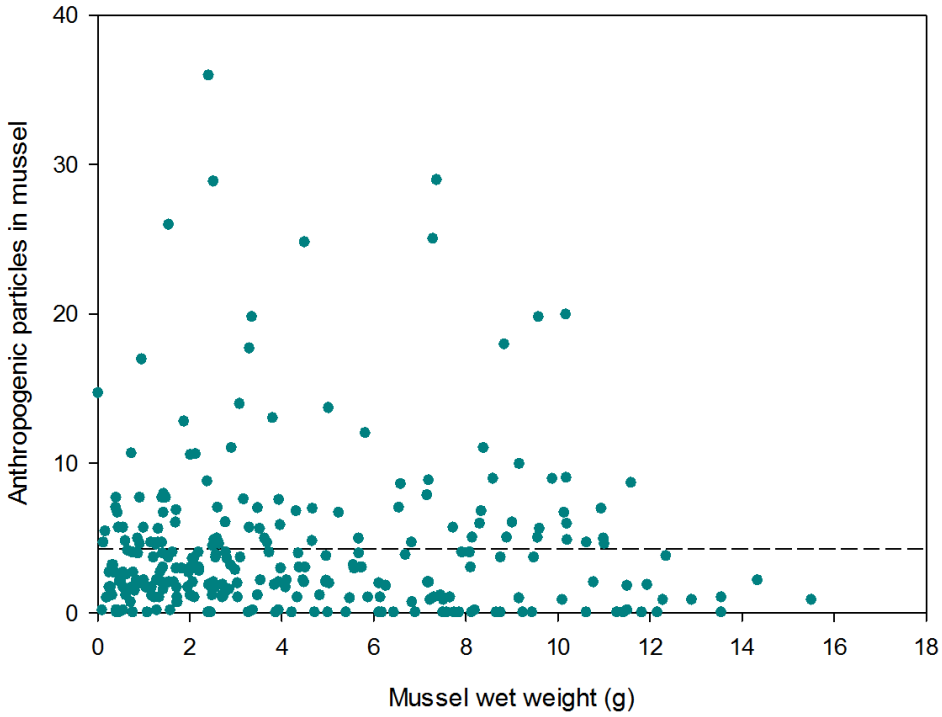

Figure 3.3. Total anthropogenic particle load per mussel plotted against mussel wet weight (g) for all mussels sampled from all sites from both years (2017 and 2018). Regression line shown as dashed line. (Linear regression, $R^2 < 0.001$, $F(1, 267) = 0.001$, p-value = 0.976).

The size and shape of anthropogenic particles also appears to influence their uptake into mussels. There were significant differences in the sizes of anthropogenic particles within mussels compared to those in the overlying seawater at study sites, with the average length of fibres in mussels significantly shorter than those in the seawater (One-way ANOVA; F2, 745 = 10.270, p-value < 0.001, Fig. 4a.). A few longer fibres were found within a number of the mussels, with the longest fibre recorded being 8.7 mm in length, suggesting occasionally the longer fibres are ingested but this does not correlate to the proportions of longer fibres available in the overlying seawater. The average size of anthropogenic fragments ingested by mussels and found in the overlying seawater samples were also significantly smaller than the particles found within the surface sediment (One-way ANOVA; F2, 54 =47.710, p-value < 0.001, Fig. 4b.). Fibres made up 67.6% of the particles within mussel samples compared to 23.4% of those present in the water samples (One-way ANOVA; F2, 140 =

11.795, p-value < 0.001, Fig. 1.). We found both high density and low density plastic polymers within the mussels, but the relative abundance of polymer types present differed from those found in the overlying seawater (Fig. 2).

We cannot make any assumptions regarding the behaviour of microplastics smaller than 50 µm here, since this was the mesh size used across all compartment samples for comparability and was the smallest size through which we could efficiently pass digested mussel tissue.

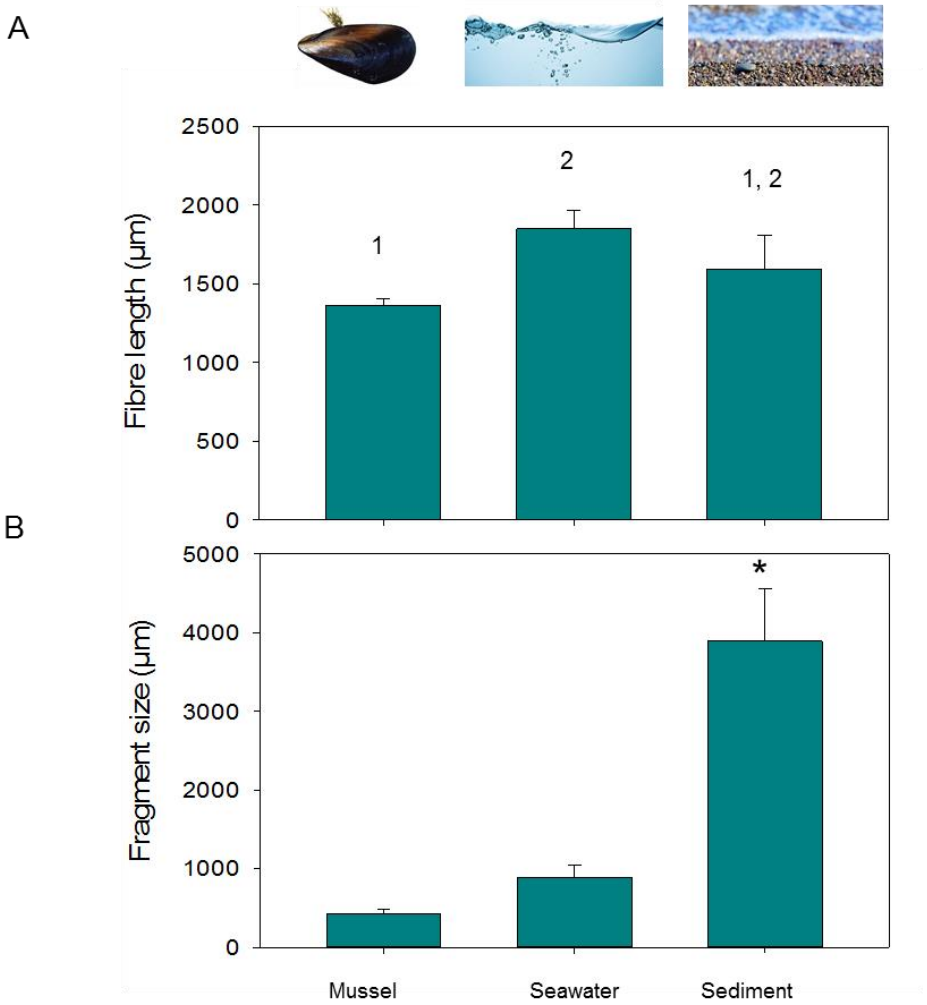

Figure 3.4. Comparisons of the sizes of the two major categories, (A) fibres and (B) fragments, of observed anthropogenic particles in samples of *Mytilus edulis,* seawater, and the surface 1 cm of sediment from coastal sites in Devon and Cornwall, SW England in 2018. Groups labelled with the same number are significantly different. (One-way ANOVA; 1) *p*-value < 0.001, 2) *p*-value < 0.001, 3) *p*-value < 0.001).

No significant relationship was found between number of particles in the overlying seawater samples and those found within mussels, but a significant positive relationship between number of particles in mussels and particles in their surrounding surface sediment (p-value = 0.031) (Fig 5a & b). Whilst clear differences are present, the proportion and the size range of fibres and the composition of the polymer types of these particles found within mussels to more closely reflect those found in the intertidal surface sediment compared to those found in the surface seawater (Figs 1, 2 and 3). Small microplastic particles have been reported to have a lower rise velocity than large particles, resulting in greater susceptibility to vertical transportation (Reisser *et al.*, 2015). This may result in smaller particles remaining suspended within benthic water for a relatively longer period of time, increasing likelihood of encounter and uptake. Particle shape has also been shown to impact vertical transport and longevity of submersion of particles (Ballent *et al*., 2013), with films and "filaments" particularly susceptible to submersion by surface turbulence.

Our findings contrast slightly with those reported by Qu *et al.* (2018), who found significant correlations between the abundance of surface seawater microplastics with mussel microplastic loads at sampling sites on the coast of China (coefficient of determination R2 values between 0.44 – 0.75 were presented to support this relationship) and similar compositions of polymer types in the mussels and the overlying seawater. Sediments and beach debris were not sampled in the Qu *et al*. (2018) study. This disparity may be due to differences in the particle characteristics of the sea surface microplastics between the two studies sites. We observed a much higher proportion of microplastic fragments in our seawater surface tows (47% of sampled particles) compared to the Qu *et al* (2018) study where fibres made up 90% of the microplastics in their seawater samples. Fibres dominate in the mussels in both studies, however, suggesting they are potentially more bioavailable to these benthic filter feeders. Since microfibres are mostly modified cellulose (Rayon) this likely drives the similarity in polymer types between seawater and mussel microplastics in the *Qu et al* (2018) study and explains the different relationship that we observe here when other polymer types are present in the overlying seawater. Differences in the habitat structure and/or

coastal hydrodynamics of the regions sampled may also play a role in between site differences in this relationship.

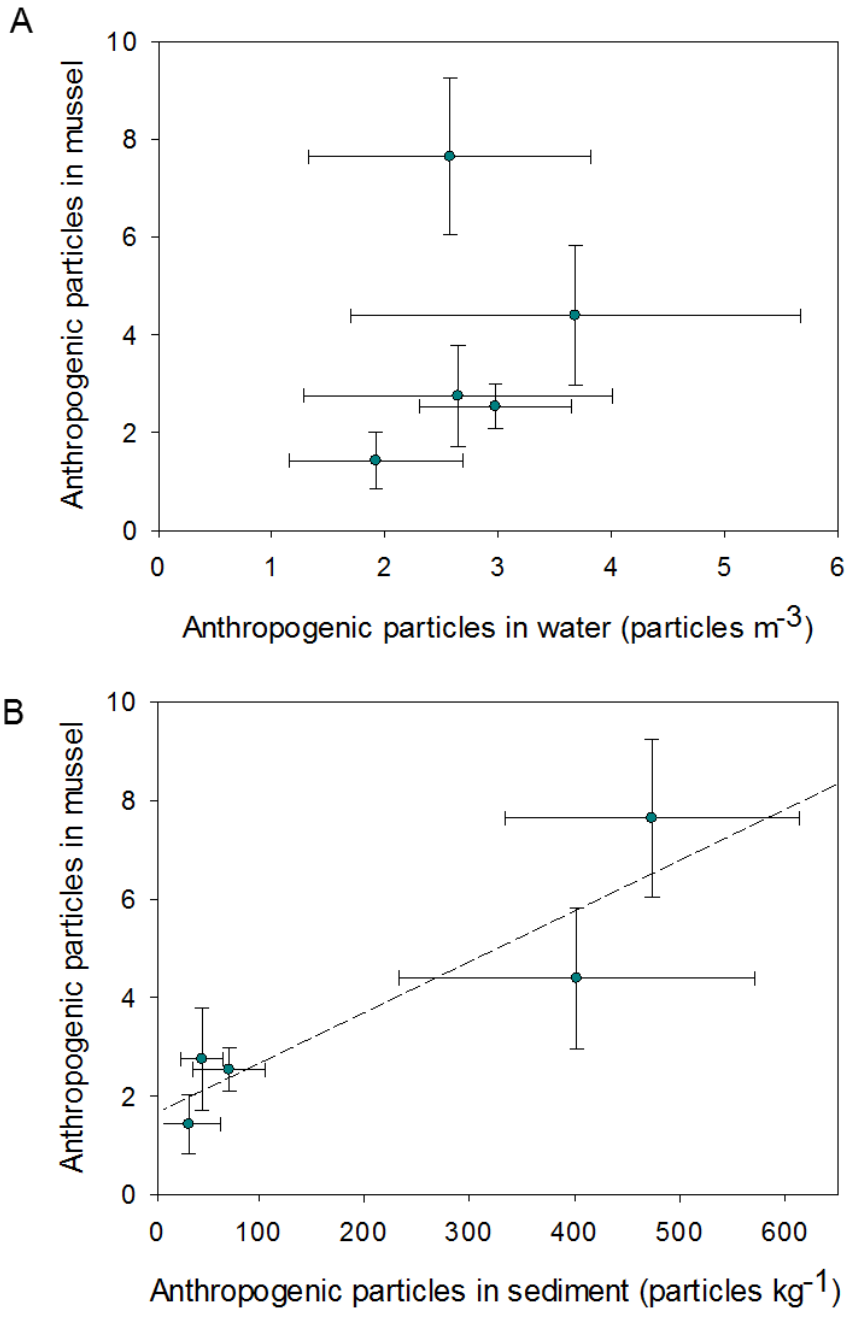

Figure 3.5. Examining the relationship between the number of anthropogenic particles in *Mytilus edulis* with those in (A) the overlying seawater and (B) the surface 1 cm of sediment at coastal sites across Devon and Cornwall, SW England. Regression line shown as dashed lines. (Linear regression, $R^2 = 0.832$, $F(1, 3) = 14.870$, p-value = 0.031).

The differences in both the size range and polymer composition of the plastics found within the mussels compared to their overlying seawater and surrounding beach sediments, suggest that uptake of microplastics into mussels may not always directly proportional to what is in their surrounding environment. It is likely that both environmental and biological partitioning of microplastic particles and the selective feeding ecology of this species is responsible for the under-representation of certain polymer types and particle sizes within the mussels. Bivalves have feeding mechanisms which enable them to discard larger particles as psudeofaeces prior to ingestion (Defossez and Hawkins, 1997). The capture of particles by feeding structures in suspension feeders such as mussels is the product of particle encounter rates and retention (Shimeta and Jumars, 1991). It is likely that a range of factors influence mussel encounter rates with particles within their immediate environment, including particle behaviour in the water column and small scale hydrodynamics. Fibrous particles may have a greater tendency for entanglement within complex feeding structures and potentially even be retained for longer periods within the gut once ingested (Murray and Cowie, 2011; Kolandhasamy *et al.*, 2018). Preferential retention of certain shapes of particles may then indirectly influence the types of polymers found within M. edulis, since the majority of fibres in our samples were cellulose. Some polymer types were under-represented or totally absent in the mussels compared to overlying seawater or surrounding sediment. This should be taken into consideration when using mussels as bioindicators of plastic pollution, since microplastic particles and polymer types that may pose a risk to other biota with differing feeding modes might be missed if this were the only monitoring tool used.

Chapter 4

Discussion - The challenges of studying microplastic dynamics in marine ecosystems.

The work towards this thesis demonstrates how microplastic particles in the marine environment can partition differently within the water column and between sea surface, benthos and a filter feeding organism according to the particle properties (size, shape and polymer type). This is likely to be important in understanding the risks that microplastics pose to marine biota, however this work also highlighted a number of technical challenges in asking more nuanced and biologically relevant questions about microplastic pollution of marine ecosystems. Here I discuss some of the challenges and recent developments of the sampling and analytical techniques applied in the field of marine plastic research.

The most common method of sampling microplastics in water is the use of neuston, manta, or plankton nets; in a recent review of sampling methods 58 % of studies used nets (Prata *et al.*, 2019; Stock *et al.*, 2019). But how samples are collected can influence results and what is found. A comparison of particle capture using plankton net sampling and via filtration of whole water samples found that not only did whole water sampling (1 L jars of seawater) collect over three orders of magnitude more microplastic particles per volume of water than a 335 µm neuston net, and a higher proportion of small microplastic (100 µm - 1.5 mm), but also a greater proportion of non-fibrous plastic (Barrows *et al.*, 2017). Covernton *et al* (2019) show that reported microplastic concentrations decrease with increasing net mesh size. Showing that not only does use of a net affect the numeration of plastics collected but also the types of particles collected. Some disparity exists between the type of net employed, with AVANI trawls collecting a greater abundance of particles in all size fractions compared to manta or DiSalvo neuston nets (Eriksen *et al.*, 2018). For sampling small microplastics, the use of nets can also result in non-negligible levels of contamination due to the materials used in their manufacture and how they are applied (Lenz and Labrenz, 2018). This could be avoided by taking whole water samples or by using an alternative

filtration device such as the pump-based apparatus designed by Lenz and Labrenz (2018) (Prata *et al.*, 2019).

The sampling method applied should be based on the questions being asked. A small-volume whole water sample may pass through fine mesh and allow analysis of the full range of microplastic particles present in the water; however the use of a plankton net might provide a more realistic assessment of the concentration of particles in the water by filtering a larger volume over a larger area (Barrows *et al.*, 2017). Although from an ecotoxicological perspective, considering the context of the organism may indicate an appropriate sampling technique. For assessing risks to zooplankton, the size range of plastic particles that are likely most important are those that can actually be ingested, and these are very small (1.7 - 30.6 µm) (Cole *et al.*, 2013). Likewise, particles < 300 µm make up a large component of microplastics commonly found in fish and invertebrates, and mussels can feed on particles down to the size of bacteria (1.2 µm), so, neuston trawls are less likely to fully capture the biologically relevant data in these instances (Ward and Shumway, 2004; Covernton *et al.*, 2019). However, for monitoring large areas which requires filtering large volumes of water and collecting samples that can be analysed quickly, the resolution of a 335 µm neuston net is likely sufficient, although there are calls to define a minimum volume of sample required to achieve representativeness (Prata *et al.*, 2019).

When looking at the potential biological impacts of microplastics, to understand the types and number of particles organisms are exposed to, sampling must occur where interactions are most likely, in areas of high productivity. Some of these regions have already been identified by comparing ocean surface plastic debris data and satellite-derived estimates of sea surface chlorophyll, a proxy for primary production (Clark *et al.*, 2016). However the plankton content of the water places a limitation on the total volume of water that can be filtered by nets, as the net mesh becomes saturated with plankton and will eventually sink (Prata *et al.*, 2019). This was apparent during collection of samples in Kongsfjorden (Chapter 2), where surface waters were so rich in biota that the plankton net could only be deployed for two minutes before sinking.

Another of the major challenges in environmental microplastics research is the isolation of microplastic particles from resilient organic material in samples; this is usually a necessary step in the preparation of samples for identification. The typical strategy is to digest or dissolve the unwanted material using hydrogen peroxide (H_2O_2), or acids or bases, such as nitric acid (HNO_3) or potassium hydroxide (KOH) (Lusher *et al.*, 2017; Prata *et al.*, 2019). However, current practices used to isolate microplastics could result in partial or complete degradation of some types of particles and influence the outcome of analyses. Whilst freezing, formaldehyde, and ethanol sample preservation techniques in mussels have been shown to cause no significant differences in the enumeration of microplastic particles or degradation of microplastics of various polymers, chemical digestion methods do have an effect (Catarino *et al.*, 2017; Courtene-Jones, Quinn, Murphy, *et al.*, 2017). Cole *et al.*, (2014) found that alkaline treatment with NaOH resulted in partial destruction of nylon fibres and yellowing of PVC granules. And HNO_3 digestion has been observed to cause melding (fusing/merging) of some PET and HDPE particles, and the destruction of nylon fibres (Catarino *et al.*, 2017). Current practices therefore might lead to an underestimation of nylon fibres in environmental samples and/or false identification results due to morphological changes to particles.

Post-sampling damage could be avoided by use of a less destructive alternative means of digestion, such as enzymes. Proteinase K, Trypsin and Corolase 7089, have all been applied effectively to achieve high recovery rates of microplastics and effectively remove biological material without altering or causing damage to the surface structure of microplastic particles (Cole *et al.*, 2014; Catarino *et al.*, 2017; Courtene-Jones, Quinn, Murphy, *et al.*, 2017; Karlsson *et al.*, 2017). However, this might not be cost-effective when processing numerous samples.

More thought should also be given to how physical and chemical alterations to the surface of particles are attained in the marine environment and could alter FTIR spectra in comparison to virgin polymer standards, and potentially impeding high quality polymer library hits (Corcoran, Biesinger and Grifi, 2009; Andrady, 2011; Costa *et al.*, 2018). In terrestrial and marine environments, microbial activity of a variety of bacteria and fungi species can cause reductions in mass, and alterations to the shape and surface structure of oil-based plastic particles

(Raddadi and Fava, 2019). And exposure to artificial seawater for 6 - 8 weeks has been shown to cause cracking in the surface of PE beads and alteration of FTIR spectra (Costa *et al.*, 2018). These spectral differences were associated with the development of new oxidized groups (the formation of a carbonyl group which have been used as a marker for oxidisation of PE) (Costa *et al.*, 2018). Physical and chemical degradation processes occur in tandem; in beach plastic, where mechanical erosion has been shown to be responsible for most of the surface textures of particles, linear fractures and particle edges tended to contain oxidation products (Corcoran, Biesinger and Grifi, 2009). Fractures created by mechanical erosion create favourable loci for chemical weathering processes, the resulting oxidation products impact spectral analysis by causing increases in the peaks in the lower wavenumber region of the FTIR spectra (Corcoran, Biesinger and Grifi, 2009).

Natural particles and fibres are difficult to distinguish from plastics, and visual identification is less achievable when particles are very small and numerous. Recent advancements in the spectral analysis of particles by FTIR and Raman spectroscopy has allowed far greater confidence in the identification of particles isolated from marine samples, however methods of sample preparation and particle analysis are varied and lack standardisation (Silva *et al.*, 2018; Primpke, Dias and Gerdts, 2019). Visual selection as the current standard protocol is arguably subjective and prone to human bias, and a automized and standardised protocol that avoided manual handling could be a more credible method of identification (Primpke, Lorenz and Gerdts, 2017). The arrangement of laboratory equipment can illuminate particles differently, changing their appearance and different coloured particles can vary in visibility. But, although colour information can be biased, this still remains important information in a biological context as in some cases colour can influence likelihood of ingestion (Hartmann *et al.*, 2019).

Spectral analysis of a larger number of particles and small particles is now possible by performing scans of whole areas of filters using a focal plane array (FPA) detector (Imaging FTIR) rather than point scanning individual particles (Primpke, Lorenz and Gerdts, 2017; Haave *et al.*, 2019; Primpke, Dias and Gerdts, 2019). This has been achieved by developing refined protocols for sample preparation to facilitate spectral analysis by FTIR spectroscopy, and

analysis of the resulting data by an automated image analysis script (Python software) (Primpke, Lorenz and Gerdts, 2017; Primpke, Dias and Gerdts, 2019). This approach can now be used for both microfibres and fragments, and allows the number of particles, the constituent polymers, and particle shape/size to be assessed without the need for visual identification. This method allows synthetic particles to be more easily differentiated from other small, naturally occurring particles while avoiding some human error and subjectivity. This protocol would have been applicable to analysis of the black fragments in deep water samples from Kongsfjorden in Chapter 2 where the sheer abundance of small particles in samples made analysing a sufficiently representative sub-sample not possible by conventional means. And many particles could not be visually identified due to the similarity with natural particles, a similar case to the natural coal fragments encountered by Bergmann, Wirzberger, *et al* (2017).

Ultimately, determining the biological consequences of microplastic pollution is a major goal of environmental plastics research, and the work for this thesis has highlighted how understanding particles characteristics and dynamics will play a role in this process. While some recent publications have begun to use automated methods for analysis of microplastic particles in sediment, seawater, waste-water, and arctic sea ice, these methods have not currently been applied to samples of organisms (e.g. Peeken *et al.*, 2018; Haave *et al.*, 2019; Primpke, Dias and Gerdts, 2019). Application of these methods to biota could help to answer some interesting questions relating to the potential ingestion of the smallest size fractions of plastic particles. However, challenges still remain in the development of these methods to improve the quality of results and positive identification, particularly for the smallest plastic particles and nanoplastics (Mintenig *et al.*, 2018). Nanoplastic particles have been observed in the marine environment but remain elusive and are a relatively unknown factor in marine plastic pollution (Halle *et al.*, 2017). These particles might be some of the most relevant to ecotoxicology studies as they are more likely to pass into tissues and accumulate, therefore understanding how these particles behave in the marine environment would be a major step forward in the field of marine plastics research.

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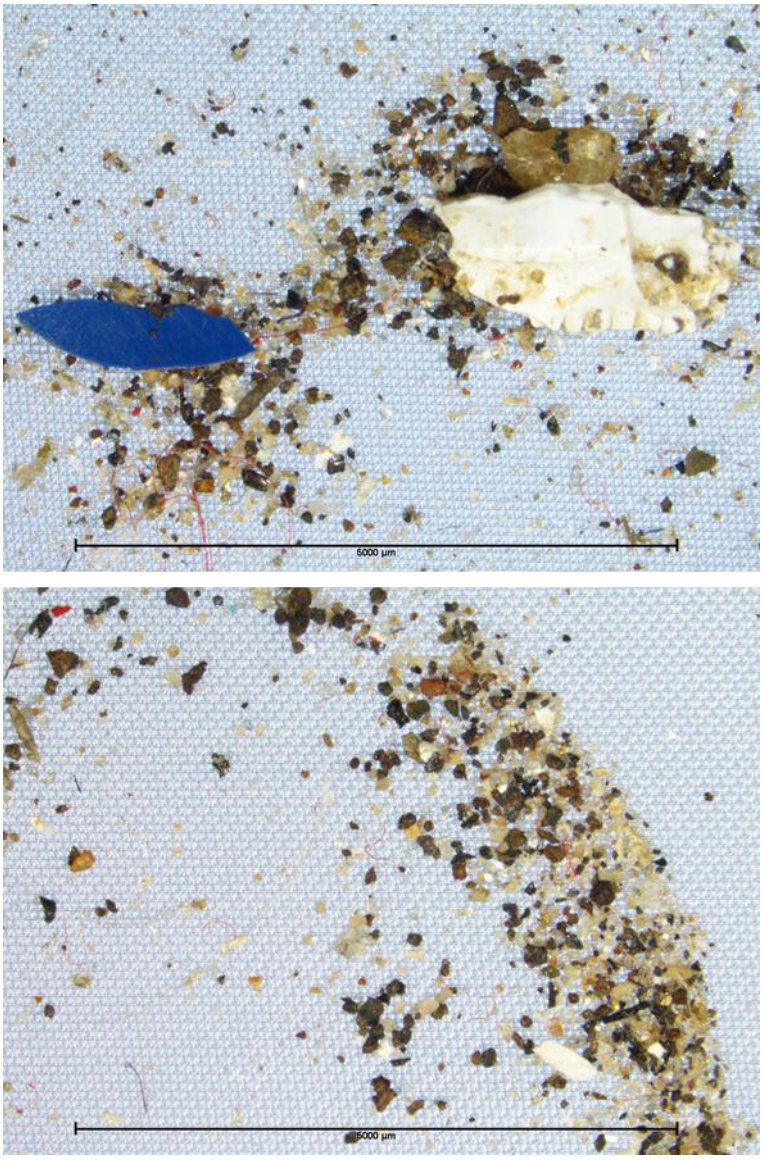
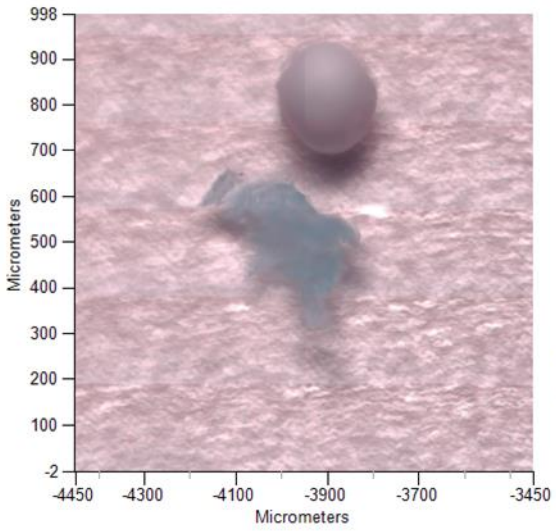
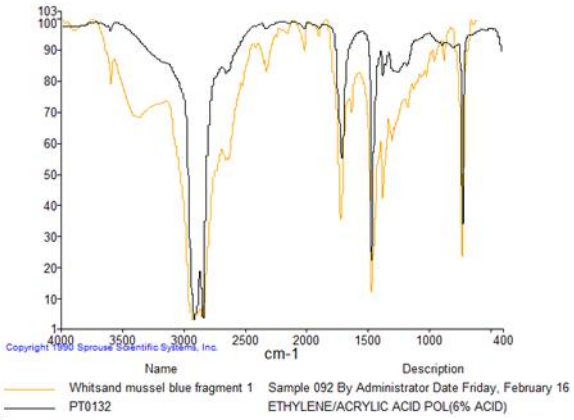
Appendices

Appendix 1.



A1.1. Sample photographs of filtered material from 160 m depth at sampling location KB2 in Kongsfjorden, Svalbard.





Search Score	Search Reference Spectrum Description
0.803865	POLYETHYLENE, OXIDIZED ACID NUMBER 15 MG KOH/G
0.804454	BBP33-3, CORO 703PE, IRS, BACK
0.809555	BP33-3, CORO 703PE, PLASTICIZER, CHCL3
0.810269	DICHLOROISOCYANURIC ACID SODIUM SALT
0.811185	BBP1-1, MONTA 250, PLASTICIZER, CHCL3 T
0.821598	POLYETHYLENE, OXIDIZED(HIGH MW)
0.825032	1-OCTANETHIOL
0.830776	NICOTINIC ACID
0.831342	1-CHLOROHEXADECANE
0.865204	ETHYLENE/ACRYLIC ACID POL(6% ACID)

A1.2. Sample µFTIR spectra, showing results for a blue fragment found in a mussel *Mytilus edulis*, from Whitsand Bay, Cornwall, U.K.

Appendix 2. The following pages show Chapter 3 as it appears published in *Marine Pollution Bulletin,* Vol. 146, pages 125 - 133. June 2019. DOI: 10.1016/j.marpolbul.2019.05.041.

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Particle characteristics of microplastics contaminating the mussel *Mytilus edulis* and their surrounding environments

Nicholas Scott[a], Adam Porter[a], David Santillo[b], Holly Simpson[a], Sophie Lloyd-Williams[a], Ceri Lewis[a,*]

[a] College of Life and Environmental Sciences: Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, United Kingdom.
[b] Greenpeace Research Laboratories, Innovation Centre Phase 2, University of Exeter, Rennes Drive, Exeter EX4 4RN, United Kingdom.

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ABSTRACT

We investigated the environmental partitioning and particle characteristics of macro-, meso- and microplastics and their uptake into the mussel, *Mytilus edulis*. Sediment samples, overlying seawater and mussels from 9 intertidal locations in the South West of England were analysed for abundance and type of microplastic. Micro- and mesoplastic-like particles were found in 88.5% of the 269 mussels sampled, ranging from 1.43 to 7.64 items per mussel. Of these plastic particles, 70.9% were identified as semi-synthetic (mainly modified-cellulose). Mussel microplastic abundance, but not polymer type, was correlated with that of their surrounding sediment, but not with sea-surface microplastic concentration or mussel size for our study sites. We found significant differences in the relative abundance of polymer types and particle sizes between seawater, sediment, and mussels, with mussels over-representing modified-cellulose fibre abundance but under-representing polyvinyl. Mussels contained significantly smaller plastic fragments than their surrounding sediment and shorter fibres than their overlying seawater.

1. Introduction

There has recently been a dramatic rise in public awareness, policy and scientific focus on plastic waste, particularly in single-use consumer products and the role of microplastic as an environmental contaminant. Between 4.8 and 12.7 million metric tonnes of plastic are thought to enter the marine environment each year (Jambeck et al., 2015), resulting in an estimated 93–236 thousand metric tonnes of microplastic particles floating on the sea surface (van Sebille et al., 2015). Plastic pollution is a global issue, with macro and microplastics now known to be present throughout both freshwater and marine ecosystems from the Arctic, to the tropics and coral reefs, and the deep sea (Courtene-Jones et al., 2017; Cózar et al., 2017; Hall et al., 2015). The definition of microplastic debris was originally arbitrarily proposed as any plastic particle < 5 mm, (Arthur et al., 2008) but it has recently been suggested this should be re-defined as particles 1 to < 1000 μm, with particles 1 mm to 10 mm now being referred to as mesoplastic (Hartmann et al., 2019). Plastic debris comprises a complex mixture of particles which are often categorised by visual characteristics such as size, colour, and shape, and is a relatively diverse pollutant, covering a wide range of sizes and shapes from larger litter items down to the nano- scale, and a range of different buoyant and non-buoyant polymer types (Hartmann et al., 2019). Micro- and mesoplastic particles fall within the size range of the optimal prey species for many animals at the base of the marine food web (Galloway et al., 2017) with increasing evidence of their ingestion by a wide range of species from zooplankton (Desforges et al., 2015) to marine mammals (Nelms et al., 2019). This combined with their prevalence and persistence throughout marine ecosystems has raised concerns globally over their potential impacts to marine species.

Globally, coastlines are diverse habitats supporting an abundance of ecologically and economically important marine species. Coastal microplastic pollution has been shown to vary by region and is dependent on a wide variety of factors such as oceanic currents, local tides and geography (Jambeck et al., 2015), but typically microplastic concentrations are high, likely due to the constant land-based input. Although plastic pollution is ubiquitous in the marine environment and can travel long distances from its sources, localised sources such as wastewater effluent and poor waste management from coastal urban populations contribute a significant component of coastal microplastic pollution (Graca et al., 2017; Jambeck et al., 2015). Hence the risk of biological uptake of microplastics in coastal regions is thought to be

* Corresponding author.
E-mail address: c.n.lewis@exeter.ac.uk (C. Lewis).

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relatively high (Clark et al., 2016; Graca et al., 2017). Whilst data on the sea-surface distribution and abundance of microplastics has increased greatly in recent years, our understanding of the movement of plastic particles away from the surface, through marine ecosystems and their ultimate fate in the marine environment remains limited. Processes such as biofouling, ingestion and subsequent incorporation into faeces, and eventual aggregation with organic matter (Zhao et al., 2018), all influence the buoyancy of plastic particles (Galloway et al., 2017) leading to the recent understanding that most plastic eventually sinks to the benthos (Koelmans et al., 2017). Hence, benthic sediments may be a major sink for plastic particles (Kaiser et al., 2017; Porter et al., 2018; Woodall et al., 2014). Along the coastline, where many benthic species feed, particles may also be re-suspended by turbulent currents and bioturbation, potentially keeping these microplastics bioavailable to benthic feeders. Microplastics are known to be readily ingested by a range of marine species including pelagic and benthic fish and invertebrates, hence benthic coastal species may be at greater risk from plastic contamination (Graca et al., 2017; Halstead et al., 2018; Lusher et al., 2013; Rummel et al., 2016). Understanding the local factors that influence biological uptake of microplastic by coastal benthic species is critical to being able to assess the risk that this pervasive pollutant poses to these important ecosystems (Seitz et al., 2014).

The mussel, *Mytilus edulis*, is a keystone coastal species with important roles in ecosystem functioning; including habitat formation for diverse benthic communities (Joint Nature Conservation Committee, 2008) and nutrient recycling. They play an important role in benthic-pelagic coupling by removing large quantities of suspended organic matter from the water by filter-feeding, and through the production of faeces and pseudofaeces (Ward and Shumway, 2004) and process large volumes of water; for example under optimal algal conditions a 21.5 mm sized mussel will filter an average of 15 mL min^{-1} (Riisgård et al., 2011). Coupled with their wide geographical range and low metabolic transformation rates, these traits make mussels useful in monitoring programmes as effective small stationary water samplers for many potential pollutants and dissolved chemical contaminants. The relationship between the level of waterborne contaminants and bivalve tissue concentrations is well established, for example in the NOAA Mussel Watch Programme which monitors over 150 organic and inorganic contaminants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), the pesticide dichlorodiphenyltrichlorethane (DDT) (National Oceanic and Atmospheric Administration, 2018). It has been suggested by a number of studies that mussels can also be useful biomonitoring tools for evaluating environmental microplastic pollution (Beyer et al., 2017; Brate et al., 2018; Li et al., 2019), however the properties of particles within the definition of “microplastic” are complex and hence their uptake into biota from the environment may not follow the same relationships or behaviours as dissolved chemicals and or their derivatives.

Microplastic uptake by mussels is well established, both in laboratory studies (Browne et al., 2008; Van Cauwenberghe et al., 2015) and in their natural habitats and may occur by ingestion or adherence to tissues (Kolandhasamy et al., 2018; Qu et al., 2018), with numerous studies now reporting microplastic contamination of wild mussels (De Witte et al., 2014; Li et al., 2018; Li et al., 2016; Phuong et al., 2018; Qu et al., 2018; Santana et al., 2016; Zhao et al., 2018). Trophic transfer of microplastics ingested by mussels has also been demonstrated under laboratory exposure scenarios, providing a route through which microplastic particles can be accumulated and enter the food chain (Farrell and Nelson, 2013). Mussels are also economically important food species, accounting for more than a third (roughly 470 thousand tonnes) of production by weight of the aquaculture industry in the European Union (Eurostat, 2016). Hence microplastic ingestion by mussels is of additional concern for its human health implications in a species which we consume whole without removing the guts (Van Cauwenberghe and Janssen, 2014).

Here, we investigate the environmental partitioning and particle characteristics of microplastics isolated from within mussels, with those of the micro- and mesoplastic particles of the mussels' immediate environment, via surveys of 10 mussel populations at 9 locations across the South West coast of the United Kingdom (U.K.). We include an assessment of the larger beach macroplastic debris at each location to assess whether there is any similarity in composition between the larger litter items and smaller microplastic items of beach plastic debris for each site. Understanding the environmental partitioning of the different types of plastic contamination across seawater and coastal sediments with its uptake in benthic mussels is key to assessing the risk that microplastic pollution poses to their ecological functions and their human consumers, as well as assessing their application as biomonitoring tools for microplastic pollution.

2. Materials and methods

2.1. Site selection

Sampling took place at 9 locations on the South West coast of U.K. during the August–December period in 2017 (mussels only) and 2018 (seawater, sediment and mussels, see SI Fig. S1 for a map of the locations and their latitude and longitude). Crooklets beach, Barricane beach, Constantine Bay, and Port Gaverne were sampled in 2017; Starcross, Yelland Quay and Trebarwith Strand were sampled in 2018. Torquay and Whitsand Bay were sampled in both 2017 and 2018. The sampling sites are mostly rocky shore beaches, with the exceptions of Starcross and Yelland quay which are estuarine habitat on the river Exe and Taw estuaries, respectively.

2.2. Water sampling

Surface seawater was sampled in triplicate for each site using a 53 µm plankton net, towed through surface water for 3 min within 10 m of the waterline, at a minimum depth of 25 cm to allow full submersion of the net. All other samples were filtered to 50 µm so as to have a consistent limit of detection for all environments sampled and hence make our results comparable across sediment/mussel/water compartments. GPS coordinates were recorded at the start and the end points of each trawl (Garmin GPSMAP® 78s) to calculate the distance of the trawl. The contents of the net were then thoroughly rinsed into 0.5 L Nalgene sample bottles using MilliQ, ultra-pure water filtered to 0.22 µm to avoid contamination from rinse water. Samples contained suspended sediment and organic matter which was allowed to settle in the bottles, then the supernatant was then filtered through 50 µm polyamide nylon mesh (Plastok® Associates Ltd.) using a vacuum filter in a laminar flow hood to reduce atmospheric contamination. Microplastic-like particles were removed from this sediment by $ZnCl_2$ density floatation separation, using the method for sediment analysis detailed below, then filtered through the same mesh as the respective supernatant. Filters were stored in sealed square petri dishes until analysed (below).

2.3. Sediment collection and density separation

Three sediment samples were collected at each site, one from within the strand line, one from the middle of the beach, and one close to the low tide mark. Sediment was collected adjacent to the mussel beds by taking the surface 1 cm of sediment from within a 1 m^2 square quadrat with a metal trowel. Sediment samples were then stored in clean plastic sample bags at −20 °C until analysed. Defrosted sediment was placed into 1 L beakers and then into a drying oven at 60 °C overnight. From each of these samples (three per site), a further three 50 g sub-samples of dry sediment were then taken for the isolation of microplastics, resulting in a total of 450 g of analysed sediment per sampling site. Whilst

this is a relatively small amount of sediment to analyse per site, this allows the use of Sediment-Microplastic Isolation (SMI) units, custom-built according to the design and methods developed by Coppock et al. (2017), to separate potential microplastics from the sediment with a high recovery efficiency (95.8%). This technique allows better recovery of micro- and meso-sized particles. A pre-filtered (50 μm) $ZnCl_2$ solution at a density of 1.5 g cm^{-3}, was chosen as a floatation media based on its effective recovery of dense polymers. The $ZnCl_2$ sediment solution was filtered through 50 μm polyamide nylon mesh using a vacuum filter and stored in sealed square petri dishes until analysed (below).

2.4. Mussel sampling

Thirty mussels were collected from each site (269 sampled in total, mean length 41.6 mm ± SD 12.7, 29 mussels from Starcross) selected to cover a wide range of mussel sizes, positions and orientations of the mussels on the substrate and within the site. Mussels were stored in plastic sample bags, and stored in a freezer at −20 °C until dissection. All subsequent work was carried out inside a laminar flow hood to minimise airborne contamination with a clean filter paper placed in a petri dish to collect airborne contamination. Once defrosted, the width and length of the shell of each mussel was measured and then thoroughly rinsed with MilliQ to remove external microplastic contamination. Mussel soft tissue was then excised and wet weight measured. During this process samples were covered with foil to avoid airborne contamination. Mussel tissue was then digested at 70 °C oven in 10% potassium hydroxide until fully digested, up to 48 h (within the range of conditions used in previous studies, reviewed by Lusher et al. (2017). The contents of each sample were filtered through 50 μm nylon mesh (for consistency with the seawater and sediment limit of detection) using a vacuum filter. Filters were stored in sealed petri dishes until further analysis.

2.5. Beach litter survey

Large plastic items were collected within a 100 m section of the beach, from the low tide mark to the back of the beach. All visible plastic was collected within an upper time limit of 90 min and standardised to the number of participants involved. Collected items were categorised using the OSPAR guideline for monitoring marine litter on beaches (OSPAR Commission, 2010b). We removed 10% of items of each category, minimum of 1 item, for FT-IR spectrometry analysis.

2.6. Analysis of filters and FT-IR analysis

Filtered material was analysed visually using a dissecting microscope at 30× magnification. Potential microplastic particles were counted and classified by shape and colour, and 10% of each category, with a minimum of three particles, were removed and stored for spectral analysis. To account for any contamination of laboratory origin, procedural blanks were performed (6 per site for mussel, and 1 per site for water and sediment samples) that underwent the same processing as water, sediment, and mussel samples but did not contain a sample. On analysis, blank samples included only fibrous particles, which is likely airborne contamination from clothing. Mussel sample blanks contained on average 1.86 ± 0.28 black fibres, 1.62 ± 0.33 clear fibres, and 0.12 ± 0.05 red fibres. The mean number of particles for each particle category (shape and colour) across the blanks was subtracted from all data prior to data further analysis and is not included in any data presented.

Potential microplastic particles were analysed using a PerkinElmer Frontier Fourier-transform infrared (FT-IR) spectrometer. For larger pieces that could be easily handled, FT-IR analysis was carried out using a universal diamond –ATR attachment. For the majority of smaller pieces FT-IR spectra were obtained using a PerkinElmer Spotlight 400 μFT-IR Imaging System (MCT detector, KBr window) operating in reflectance mode and with a wavenumber resolution of 4 cm^{-1}. A total of 16 scans were collected, across a wavenumber range from 4000 to 650 cm^{-1}. Spectra were then processed using Perkin-Elmer's Spectrum™ 10 (version 10.5.4.738), enabling normalisation of the data and base-line correction. Polymers were identified by automated matching against commercially available spectral libraries, including Perkin-Elmer's standard Polymers Library. Only match qualities > 70% were accepted, with an average match quality of samples of 85%. Particles were photographed using the spectrometers imaging software and the lengths of fibres and fragments then measured using ImageJ 1.47v (Schneider et al., 2012). Prior to data analysis, particle categories which could not be confirmed as synthetic by μ-FTIR spectrometry, were excluded. This included “film” in which all particles examined were confirmed as chitin, and “white beads” which were all confirmed as calcium carbonate mussel pearls. Larger plastic pieces from the beach litter survey were analysed using a Cary 630 FTIR spectrometer (Agilent Technologies). Samples were prepared for analysis by removing the degraded and biofouled surface layer with a razor blade to improve the quality of the spectra. Biofilms have been shown to mask the distinct identifying peaks of synthetic polymers (Ghosal et al., 2018).

2.7. Data analysis

Data presented is based on the confirmed anthropogenic particles following FTIR analysis. Statistical analyses (ANOVA and linear regression) were performed on data corrected for contamination found in procedural blanks using SPSS Statistics 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY). Differences between total number of particles in seawater, sediment, and mussel samples were determined using One-Way ANOVA with a Tukey's post hoc test. Linear regressions were used to determine the relationship between microplastic in mussel tissue, seawater and sediment. Linear regression was also used to determine the relationship between mussel size and microplastic particle abundance. Statistical significance was accepted at p-value < 0.05.

3. Results and discussion

Microplastic contamination of seawater, coastal sediments, and mussels was evident at all of our sampling locations across the South West of the U.K. All surface seawater samples contained microplastic particles, with concentrations ranging from 1.97 to 3.38 particles m^{-3}, but with no significant differences in seawater concentrations of these particles across our study sites (Fig. 1a, one-way ANOVA, $F_{4,10} = 0.228$, p-value = 0.916). Of these floating particles, 51% were microfibres and 47% were fragments, with only 0.03% comprising microbeads. Microplastic contamination of the surface layer of intertidal sediment did differ significantly between locations (Fig. 1b, one-way ANOVA, $F_{4,10} = 4.544$, p-value = 0.024), with concentrations ranging from 33.9 particles kg^{-1} at Torquay to 402.0 particles kg^{-1} at Whitsand Bay. The majority of these particles were microfibres (93%), with only 7% being fragments, found in samples from only three of the five sites analysed for sediment. No microbeads were observed in the sediment samples analysed from our study sites.

Microplastic particles were found within 238 of the total 269 mussels sampled (i.e. 88.5% of mussels) across the 10 mussel populations studied (from 9 locations; two different populations were sampled within Torquay Bay) (Fig. 1c). Whilst seawater microplastic concentrations did not differ across sites, the particle load per mussel did differ significantly between our study sites (One-way ANOVA; $F_{9,259} = 4.018$, p-value < 0.001 Fig. 1c), with mussels from Whitsand Bay containing the highest average particle loads of 7.64 ± 1.61 particles per individual, and Torquay (harbour) the least, with 1.43 ± 0.30 particles per individual. Of these particles, 87% were microfibres whilst 12% were fragments. Only 9 microbeads were found

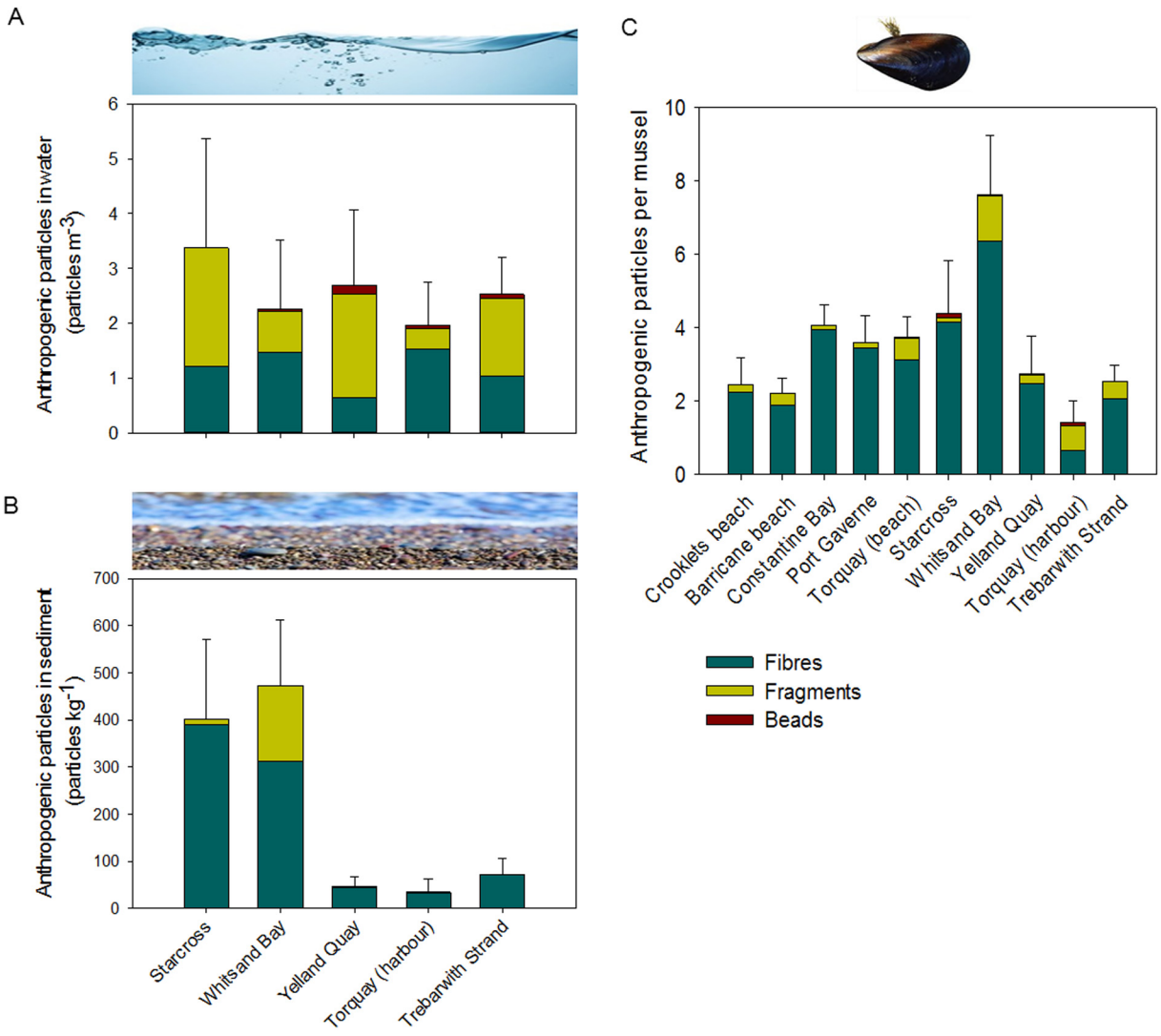

Fig. 1. The average number of microplastic-like particles, characterised according to shape found in (A) surface seawater (2018 data) (B) the surface 1 cm of sediment (2018 data) and (C) within the tissues of the mussel *Mytilus edulis* (2017 and 2018 data) at coastal sites in Devon and Cornwall, SW England. Data as mean ± standard error (limit of detection cross all samples of 50 μm).

within mussels across all sites sampled (< 1%). These numbers of microplastic particles per individual mussel are similar to the range reported in a previous study on microplastic contamination of mussels in the U.K. (Li et al., 2018) (1.1–6.4 items per individual) and are similar to those reported in China (Li et al., 2018) and Norway (Brate et al., 2018). However they are higher than the contamination levels reported for mussels in other studies from Belgium, Germany, French and Dutch coastal waters (De Witte et al., 2014; Van Cauwenberghe et al., 2015; Van Cauwenberghe and Janssen, 2014). The highest numbers of microplastics reported for mussels to date is that reported for mussels collected from a beach in Nova Scotia, Canada, where 34–178 items/individual was recorded, mostly comprising microfibres (Mathalon and Hill, 2014).

Micro-FTIR spectroscopy was conducted on 247 randomly selected particles from across the seawater, sediment and mussel samples. This analysis revealed that 33.9% of these particles were synthetic plastic polymers, mainly polystyrene, polyethylene and polypropylene (Fig. 2). Particles of natural origin, 9.3% of items analysed, and spectra with a low match quality (below 70%) were discarded from our final results and are not presented in our data. A large number of particles (56.8%), were semi-synthetic fibres comprised of modified-cellulose. Potential rubber fragments were also found in some samples but are not included in the data presented due to difficulties in generating high quality FTIR spectra from these particles. The modified-cellulose fibres were mostly black/blue or red and hence are likely to be viscose/rayon fibres from textiles, therefore we include these within our counts as these highly modified natural polymers have been included within the recent 'microplastic' definition suggested by Hartmann et al. (2019) due to their artificial composition.

This follows an emerging trend for studies in coastal areas where particles are subsequently analysed using μFT-IR or other spectral techniques such as Raman, which often find a high percentage of anthropogenic particles in seawater or ingested by marine species comprise modified-cellulose-based anthropogenic materials such as viscose or rayon (Remy et al., 2015), or natural fibres such wool or cotton (Courtene-Jones et al., 2017; Halstead et al., 2018; Li et al., 2018). For example, Brate et al. (2018) reports cellulose fibres as the dominant particle in mussels on the Norwegian coast, whilst a recent global study found that 57% of the microfibres isolated from marine samples are classified as synthetic, 12% as semi-synthetic, and 31% as non-synthetic (Barrows et al., 2018). According to the recent Hartmann et al. (2019) review, synthetic-cellulose fibres should be considered within the definition of 'plastic debris' due to their highly modified and persistent nature, however distinguishing between synthetic and natural

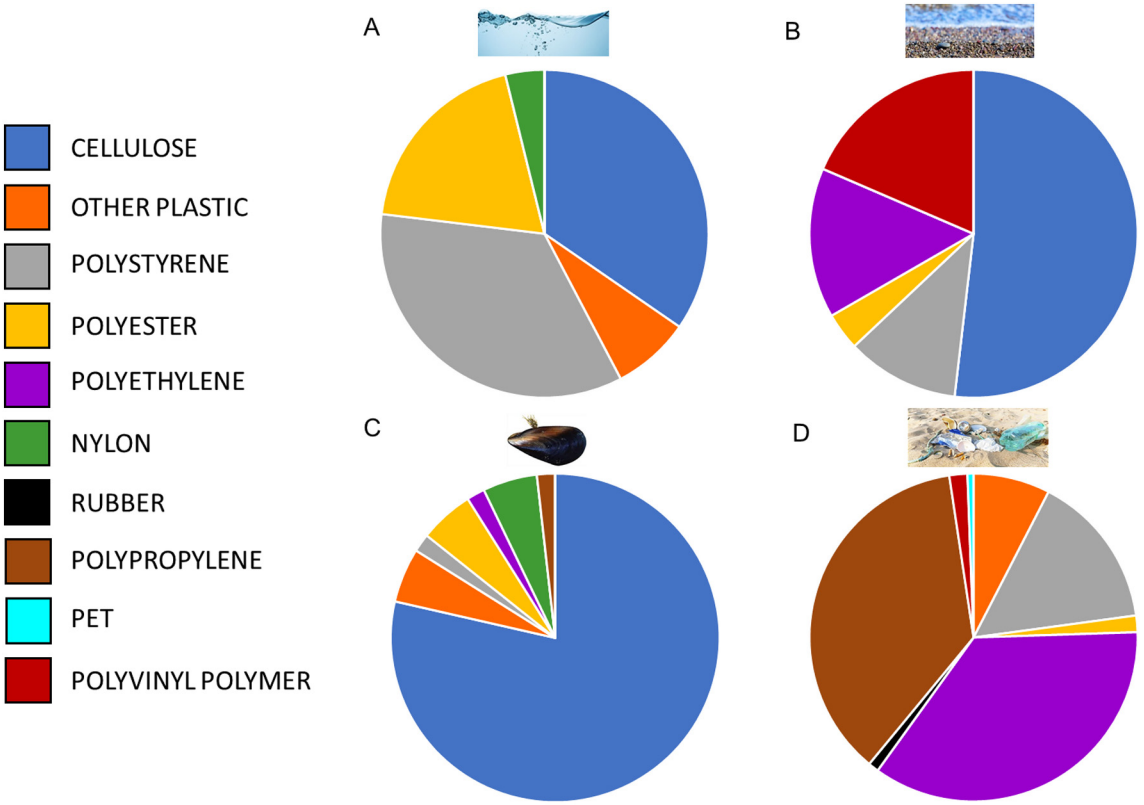

Fig. 2. Results of ATR/FT-IR spectral analysis, showing proportions of polymers of anthropogenic particles present in (A) samples of seawater, (B) the surface 1 cm of sediment, (C) within *Mytilus edulis*, and (D) macroplastic beach debris from coastal sampling sites in Devon and Cornwall, SW England.

cellulose-based fibres using currently available μFT-IR spectral libraries can be challenging, making categorising these fibres as either plastic or non-plastic particles problematic.

A variety of synthetic and semi-synthetic polymers were found across the different environmental compartments that we studied (i.e. seawater, sediment, mussels), however these were not all distributed equally across compartments, i.e. environmental partitioning of polymer types was observed which may influence what is bioavailable to a benthic mussel to ingest. For example, modified-cellulose made up significantly more of the particles found in mussels than in the overlying seawater or the beach litter at our sample sites (One-way ANOVA; $F_{3,\ 22} = 19.282$, p-value < 0.000, Fig. 2). The buoyant polymer polyester (7.5% of total) made up a significantly greater proportion of particles in the overlying seawater than those in the sediment or in the beach macroplastic items (One-way ANOVA; $F_{3,\ 22} = 5.990$, p-value $= 0.004$, Fig. 2b). There was a significantly greater proportion of polyvinyl polymers in the sediment than in mussels or the overlying seawater (One-way ANOVA; $F_{3,\ 22} = 8.039$, $p = 0.002$). Other polymers identified include polystyrene (11.0%), polyethylene (3.4%), polyvinyl-based polymers (4.2%), nylon (2.5%), modacrylic (1.7%), and polypropylene, polyacrylamide, ethylene/acrylic acid, and plasticizer (0.85% each) (Fig. 2a, b, c).

Macroplastic pollution of the strandline and intertidal zone was evident at all of our sampling locations but varied greatly in abundance from site to site. We collected a total of 7411 beach macroplastic debris items, of which 3723 items were collected from Whitsand Bay, accounting for more macroplastic items than the sum of all other locations. Trebarwith Strand was the least littered site with only 17 items collected. Macroplastic beach litter was diverse in composition but was dominated by fragmented plastic debris with pieces 0–2.5 cm and pieces 2.5–50 cm making up 44.8% and 35.0% of total collected items by number, respectively, consistent with previous beach litter studies for the U.K. (Nelms et al., 2017; Watts et al., 2017). Other items (< 5% each) were mostly consumer products such as food and cosmetic item packaging and containers, ropes, cigarette lighters, and plastic bags (for full list of items see Table S1 in Supplementary materials). We analysed 811 of these macroplastic items using FTIR, with an average certainty of 85.4% in order to compare these polymer types with the composition of the microplastic particles found at the same locations and within the mussels. Despite a large variety in litter items, the macroplastic was dominated by only two buoyant polymers, polyethylene (35.4%) and polypropylene (36.6%), representing a significantly greater proportion than found at the micro- scale (One-way ANOVA; $F_{3,\ 22} = 7.747$, p-value $= 0.001$; $F_{3,\ 22} = 20.814$, p-value < 0.001, respectively, Fig. 2d). The remaining items comprised polystyrene (15.3%), polyvinyl polymers (1.78%), polyester (1.63%), rubber (1.0%), polyethylene terephthalate (0.6%) and 'other' plastic polymers (7.6%), (Fig. 2d).

These large differences in the polymer composition of large macroplastic litter on beaches and the microplastics found in the same sediments, the nearby surface seawater and within the mussels suggests that there is no direct relationship between the two size fractions of debris at the sites tested here, i.e. the larger macroplastics litter items are not the source of the smaller items on the same beach. The local coastal topography, sediment type, and hydrodynamics, in addition to particle characteristics are all likely to play a role to produce the mix of plastic items that accumulate on any section of coastline (Zhang, 2017). The fragmentation of coastal macroplastic debris might produce particles with altered physical characteristics from the original larger items which are then influenced differently by local physical factors. Particle shape, size, and density may determine a particles position in the water column and changes to these characteristics could determine the way in which the particles are transported (Kowalski et al., 2016; Lebreton et al., 2018). Whether a particle is in suspension or settled in the sediment could determine to what degree it affected by surface currents and turbulence, wind and wave induced drift, or benthic sediment

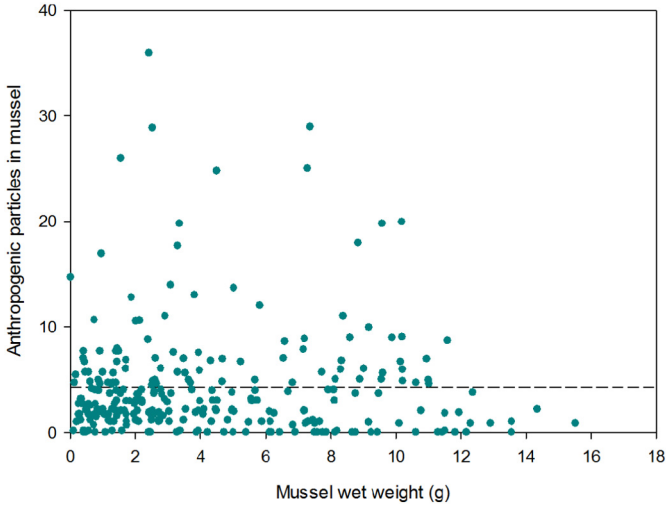

Fig. 3. Total anthropogenic particle load per mussel plotted against mussel wet weight (g) for all mussels sampled from all sites from both years (2017 and 2018). Regression line shown as dashed line. (Linear regression, $R^2 < 0.001$, $F(1, 267) = 0.001$, p-value = 0.976).

transport dynamics such as bed-load or suspended-load (Ballent et al., 2013; Zhang, 2017). Ultimately, changes to a particle's physical characteristics could result in transport away from the site of origin. This may explain why we find such a high percentage of polypropylene and polyethylene at the macro- scale, but not at micro- or meso-scales at these intertidal sites.

We found no correlation between the total number of anthropogenic particles in individual mussels and mussel wet weight (g) or any other parameter of individual size tested at the sites studied for this work (Fig. 3). This is in contrast with previous findings of Brate et al. (2018) who did find a relationship between mussel size and number of particles ingested in their study of Norwegian mussel populations. Studies in microplastic uptake often attempt to normalise their measures of plastic particles per individual by mass, following an assumption that size influences uptake rates in a similar way to respiration rates (Hamburger et al., 1983) and feeding rates (Riisgård et al., 2014). Our study suggests that this idea of scaling of microplastic uptake proportionally to size might not always hold true for *M. edulis* at this particle size range and lower concentrations of plastic contamination. Whilst mussel condition varies seasonally and hence shell length may be considered a more reliable indicator of filtration rate than tissue weight (Riisgård et al., 2014), we similarly found no relationship between shell length and microplastic uptake. Little dose response data exists for microplastic uptake for any marine species, particularly at these lower

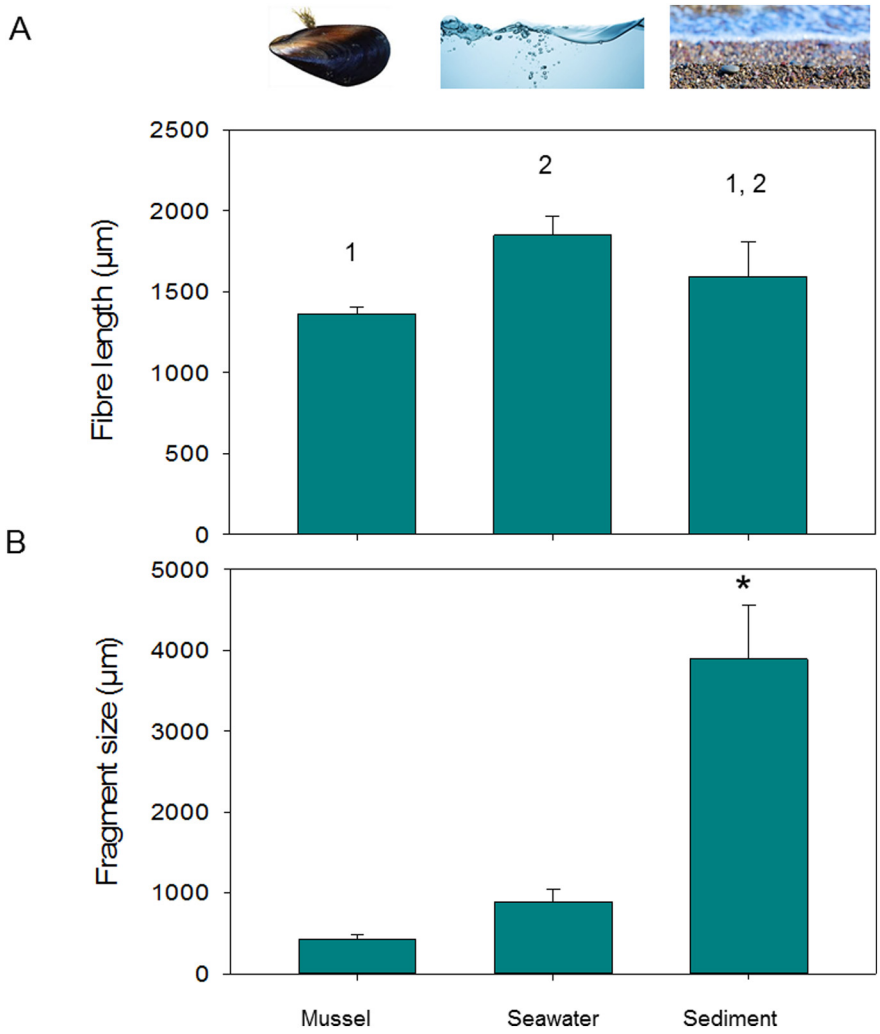

Fig. 4. Comparisons of the sizes of the two major categories, (A) fibres and (B) fragments, of observed anthropogenic particles in samples of *Mytilus edulis*, seawater, and the surface 1 cm of sediment from coastal sites in Devon and Cornwall, SW England in 2018. Groups labelled with the same number are significantly different. (One-way ANOVA; 1) p-value < 0.001, 2) p-value < 0.001, 3) p-value < 0.001).

environmental concentrations. Microplastics can also pass through guts and be egested with the faecal material (Cole et al., 2016), so may only ever be present within an individual for a short time related to the gut passage time of that individual. This coupled with the transitory nature of floating plastic debris creates a series of interdependencies when considering how mussels become contaminated with microplastics. For instance, microplastics have been found to vary by 3 orders of magnitude difference between sites only 32 km and 75 km away from a net tow within a 24 h period (Law et al., 2014) and therefore our data do not constitute a definitive view of the question at hand. This may be shape dependant with fibres potentially being more likely to be retained but the evidence supporting this idea is currently limited. Hence the dynamics of particle uptake and body load may not scale with size at these low concentrations but rather be driven by particle encounter rates influenced by localised seawater movement at microscales.

The size and shape of anthropogenic particles also appears to influence their uptake into mussels. We found significant differences in the sizes of anthropogenic particles within mussels compared to those in the overlying seawater at our study sites, with the average length of fibres in mussels significantly shorter than those in the seawater (One-way ANOVA; $F_{2,\ 745} = 10.270$, p-value < 0.001, Fig. 4a). A few longer fibres were found within a number of the mussels, with the longest fibre recorded being 8.7 mm in length, suggesting occasionally the longer fibres are ingested but this does not correlate to the proportions of longer fibres present in the overlying seawater. The average size of anthropogenic fragments ingested by mussels and found in the overlying seawater samples were also significantly smaller than the particles found within the surface sediment (One-way ANOVA; $F_{2,\ 54} = 47.710$, p-value < 0.001, Fig. 4b). Fibres made up 67.6% of the particles within mussel samples compared to 23.4% of those present in the water samples (One-way ANOVA; $F_{2,\ 140} = 11.795$, p-value < 0.001, Fig. 1). We found both high density and low density plastic polymers within the mussels, but the relative abundance of polymer types present differed from those found in the overlying seawater (Fig. 2).

We used a 50 μm mesh for our analysis across seawater, sediment and mussels here to enable a direct comparison of particles characteristics across these environmental compartments, and as such are likely missing particles present below this limit of detection. Whilst plastics are now being found at the nano-scale in the marine environment (Ter Halle et al., 2017) the sampling of open seawater with a limit of detection below 50 μm remains relatively rare and is an increasingly recognised gap in global marine plastics data. There is a trade-off when sampling surface waters of the volume of seawater that can be sampled versus limit of detection. Plankton nets of smaller mesh size clog rapidly with plankton and organic matter, and 'whole water' sampling methods tend to limit the volume of water that can be sampled to much smaller volumes. Sampling of sediments for the smaller microplastic fraction is even more challenging, particularly on beaches with fine sediment grain sizes. Hence the lower threshold of 50 μm used here was the lowest size threshold we could analyse quantitatively for reasonable sample volumes across all environmental compartments. The relationship between microplastic particles found within mussels and those in their surroundings may be quite different for particles smaller than 50 μm, however data on this size range of microplastic particles for coastal and open seawater is currently limited.

We found no significant correlation between number of particles in the overlying seawater samples and those found within the mussels at our given sites within our limit of detection, but did observe a significant positive correlation between number of particles in mussels and particles in their surrounding surface sediment (p-value = 0.031) (Fig. 5a & b). We found the proportion and the size range of fibres and the composition of the polymer types of these particles found within mussels to more closely reflect those found in the intertidal surface sediment compared to those found in the surface seawater (Figs. 1, 2 and 3). Small microplastic particles have been reported to have a lower rise velocity than large particles, resulting in greater susceptibility to vertical transportation (Reisser et al., 2015). This may result in smaller particles remaining suspended within benthic water for a relatively longer period of time, increasing likelihood of encounter and uptake. Particle shape has also been shown to impact vertical transport and longevity of submersion of particles (Ballent et al., 2013), with films and "filaments" particularly susceptible to submersion by surface turbulence. Our findings contrast slightly with those reported by Qu et al. (2018), who found significant correlations between the abundance of surface seawater microplastics with mussel microplastic loads at sampling sites on the coast of China (coefficient of determination R^2 values between 0.44 and 0.75 were presented to support this relationship) and similar compositions of polymer types in the mussels and the overlying seawater. Sediments and beach debris were not sampled in the Qu et al. (2018) study. This disparity may be due to differences in the particle characteristics of the sea surface microplastics between the two studies sites. We observed a much higher proportion of microplastic fragments in our seawater surface tows (47% of sampled particles) compared to the Qu et al. study where fibres made up 90% of the microplastics in their seawater samples. Fibres dominate in the mussels in both studies, however, suggesting they are potentially more bioavailable to these benthic filter feeders. Since microfibres are mostly modified cellulose (Rayon) this likely drives the similarity in polymer types between seawater and mussel microplastics in the Qu et al. (2018) study and explains the different relationship that we observe here when other polymer types are present in the overlying seawater. Differences in the habitat structure and/or coastal hydrodynamics of the regions sampled, as well as the abundances of plastics present and the distance to point sources of microplastics may also play a role in between site differences in this relationship. Additionally, the differing results may be the result of alternate methods of surface water sampling, Qu et al. (2018) used 5 L grab samples whereas we used plankton nets. Both methods have benefits and limitations (Barrows et al., 2017). Grab samples can capture the full range of particle sizes, but the small volume of water sampled may result in high variability between replicate samples. Plankton nets allow far greater volumes of water to be sampled efficiently, however are limited to a minimum particle size and will not capture all particles.

The differences in both the size range and the polymer composition of the plastics found within the mussels compared to their overlying seawater and surrounding beach sediments, suggest that uptake of microplastics into mussels may not always directly proportional to what is in their surrounding environment. It is likely that both environmental and biological partitioning of microplastic particles and the selective feeding ecology of this species is responsible for the under-representation of certain polymer types and particle sizes within the mussels. Bivalves have feeding mechanisms which enable them to discard larger particles as psudeofaeces prior to ingestion (Defossez and Hawkins, 1997). Indeed Kolandhasamy et al. (2018) found that the largest microplastics in their study were adhered to the foot and mantle rather than ingested and so the capture of particles by feeding structures in suspension feeders such as mussels is the product of particle encounter rates and retention (Shimeta and Jumars, 1991). It is likely that a range of factors influence mussel encounter rates with particles within their immediate environment, including particle behaviour in the water column and small scale hydrodynamics. Fibrous particles may have a greater tendency for entanglement within complex feeding structures and potentially even be retained for longer periods within the gut once ingested (Kolandhasamy et al., 2018; Murray and Cowie, 2011). Preferential retention of certain shapes of particles may then indirectly influence the types of polymers found within *M. edulis*, since the majority of fibres in our samples were cellulose. Some polymer types were under-represented or totally absent in the mussels compared to overlying seawater or surrounding sediment. This should be taken into consideration when using mussels as bioindicators of plastic pollution, since microplastic particles and polymer types that may pose a risk to other biota with differing feeding modes might be missed if this were

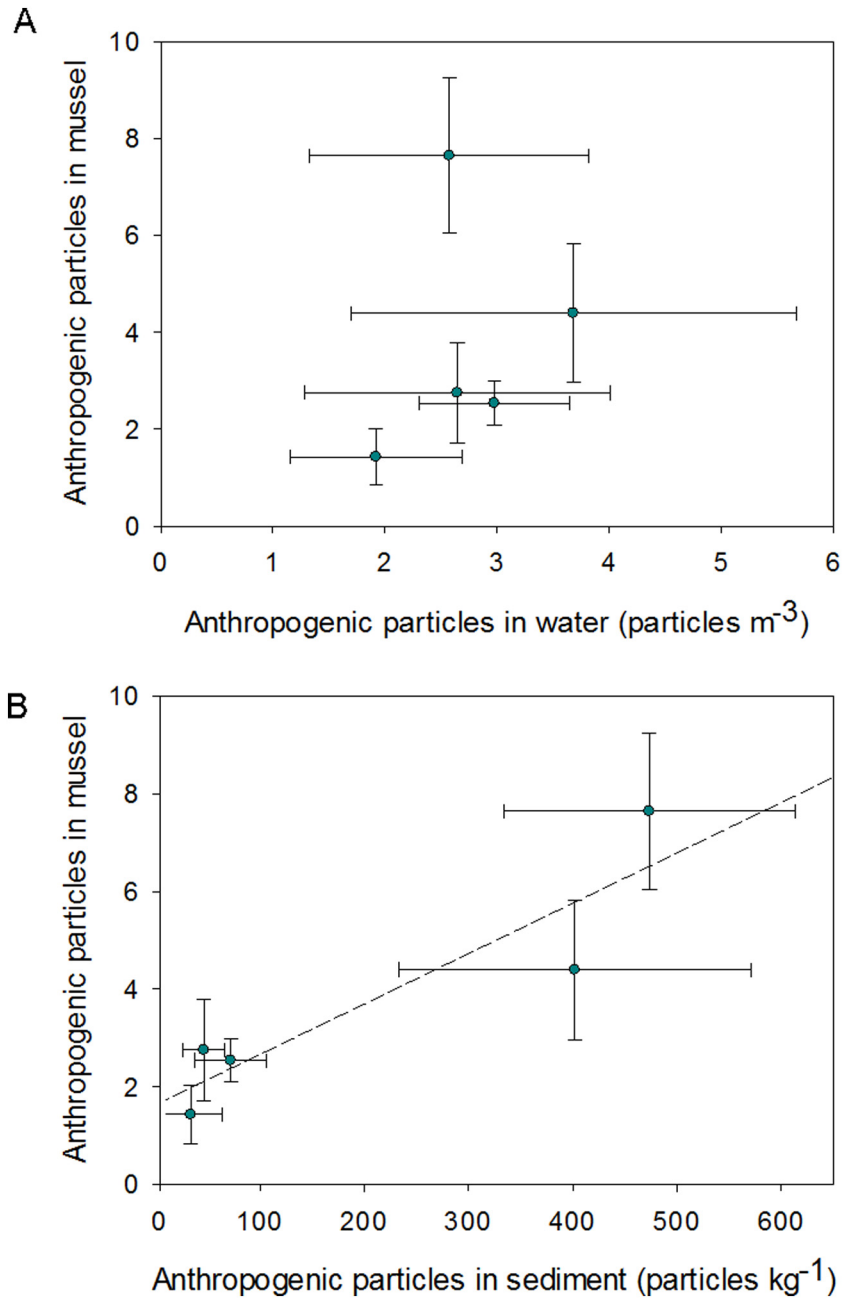

Fig. 5. The number of anthropogenic particles in *Mytilus edulis* compared to those in (A) the overlying seawater and (B) the surface 1 cm of sediment at coastal sites across Devon and Cornwall, SW England. Regression line shown as dashed lines (Linear regression, $R^2 = 0.832$, $F(1, 3) = 14.870$, p-value = 0.031).

the only monitoring tool used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.05.041.

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